General Plant Physiology

by

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Foreword by

Sir F. Gowland Hopkins, O.M.

With 44 Diagrams in Text

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FOREWORD

'LANT PHYSIOLOGY, like other branches of biology, is widening ts scope as the result in particular of the rapid development in ts experimental technique. It is making fresh contacts with other ›ranches of science, and the large body of knowledge which it ıas now won requires more sectional treatment than was necessary ›ut a few years ago. Many text books devoted to the subject loubtless exist, but the appearance of another of the right kind s at the present moment timely, and should be welcomed. In ny opinion Mr. Barton Wright's book is of this right kind.

In the case of a rapidly growing branch of knowledge, an en-irely fresh book has advantages over a classical text book "brought ıp to date." In the latter the presentation of new knowledge is ›ften hampered by the rigidity of the original scheme. The new s apt to appear as patch-work on the old.

The scheme and scope of the present book are based upon he knowledge of to-day, and in five hundred pages it presents hat knowledge with remarkable completeness. To me, at least, t seems that nothing of significance is missing. The sections into which the subject is divided for presentation are well chosen and ırranged in a logical sequence, and what is very convenient for he reader who consults the book with a special aim, each section s to a large extent complete in itself. In illustration I may refer o two such sections, the one dealing with the classical and central problem of plant physiology, namely photosynthesis; and the other concerned with very new knowledge, the influence of hormones on growth.

The former subject is treated, as it should be, historically, ›ut brought fully up to date, while all the available facts concern-ng the latter are carefully marshalled. In all sections proven acts are carefully distinguished from speculations.

I commend this book to all students of the subject, and to those eachers who, like myself, have been waiting for a recent and reliable account of its progress.

F. GOWLAND HOPKINS

October 1937

TO MY FRIEND

DR. F. KIDD

AUTHOR'S NOTE

'HIS text is meant as a general survey of plant physiology for rst and second year University students. It does not pretend to ive the most recent information on any one particular branch; ather has the aim been to discuss the fundamental principles of ıe subject. A large number of different texts have been consulted nd references to the literature from 1900 onwards are given on ach page.

There only remains for me the very pleasant task of thanking umerous friends and colleagues for their help. To Sir F. Gowland Iopkins I am more than grateful for writing the foreword, while) Miss M. Jockel and my colleague Dr. J. B. Hutchinson I owe deep debt of gratitude for the unselfish way in which they ıouldered the wearisome task of reading and re-reading the 'hole of the proofs. I should also like to take this opportunity of ıanking Miss Jean McDougal for reading the manuscript.

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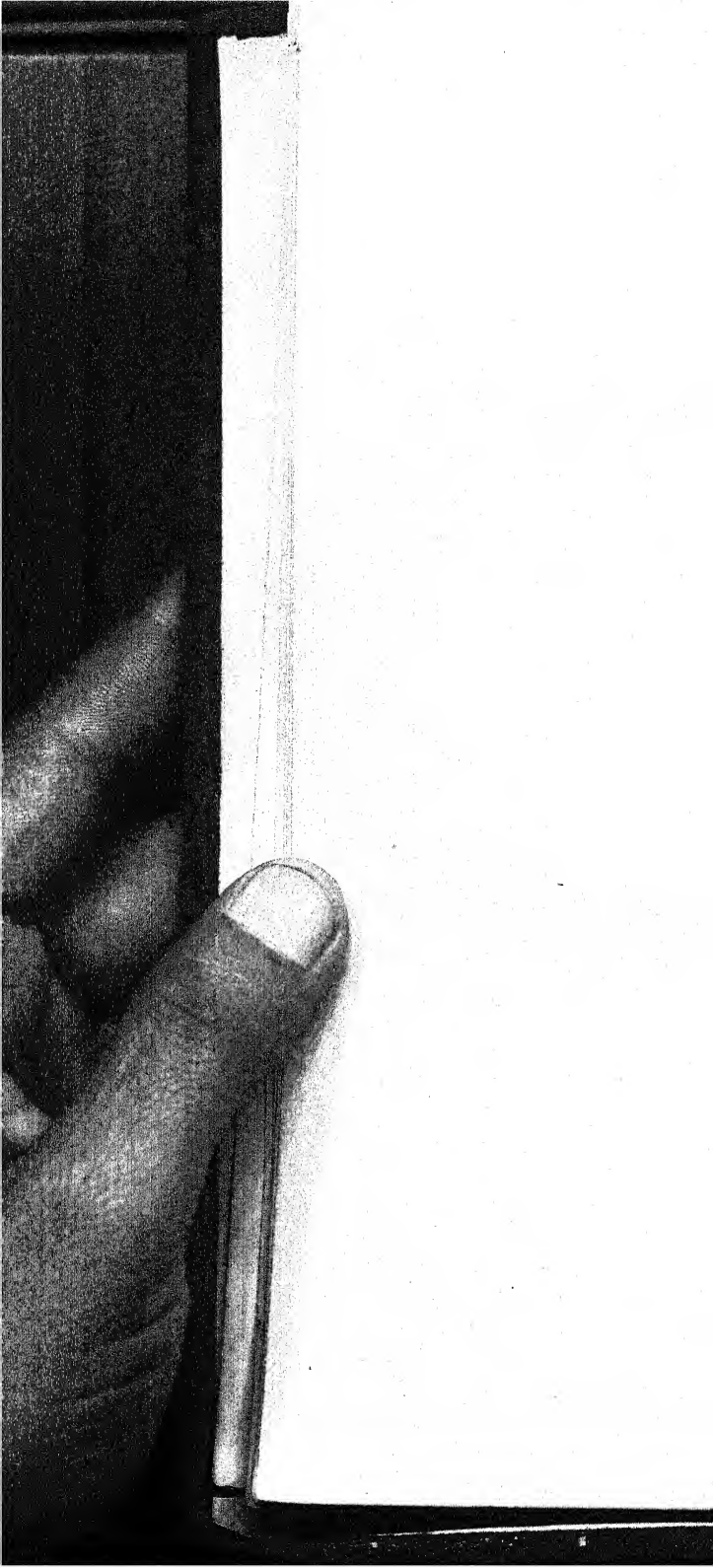
PART I

THE GENERAL PHYSIOLOGY OF THE CELL



CHAPTER I

THE SCOPE OF PHYSIOLOGY

PHYSIOLOGY is the branch of science which deals with the special form of activity we term *life*. It endeavours to explain the phenomena manifested by living organisms, and its ultimate goal is to explain them in terms of physics and chemistry. Since physics and chemistry are quantitative sciences, physiology is a particularly important branch of biology; for the physiologist cannot be content with mere verbal description, in the same way as say the morphologist or taxonomist, but must reduce his data wherever possible to a quantitative basis. Actually physiology rests upon a tripod of three sciences: anatomy, physics and chemistry. A knowledge of structure is necessary to the physiologist, for he must be acquainted with the various organs of the living body he is studying, so that he may understand their function in the general economy of life. It is only by studying a living organism from the physiological standpoint that a proper conception can be obtained of how it lives, moves and maintains its being in the general struggle for existence to which all living organisms are subjected.

For the sake of convenience, biologists have divided living things into two great and comprehensive groups, animals and plants. Although, generally speaking, it is a simple matter to distinguish an animal from a plant as far as the higher forms of life are concerned, the distinction becomes a matter of difficulty in the lower scales of life (cf. Euglena).

There is at present no satisfactory comprehensive definition of a living organism, but living beings have certain definite characteristics. All living things are capable of movement, whether it be the rapid flight of a bird or the slow unfolding of a flower. Moreover, the impulse of movement comes from within and is not like the movement of dust or sand before a storm. All living things feed. Matter is taken up from without and altered chemically, and from these chemical changes energy is released for growth and movement. Lastly, life reproduces itself. All living things are developed from a parent or parents. Life can only arise from pre-existing life, and as far as our present knowledge is concerned, there is no such phenomenon as "spon-

taneous generation" of living beings. It is the province physiologist to study these phenomena and to offer explana The field of work is, however, so enormous that the scie physiology subdivides naturally into plant and animal physi Here we are more particularly concerned with the physiol plants, but it will be necessary to employ examples to illu special points from the animal side of the subject.

The living plant is not a mass of chemical substances, the seat of active chemical and physical processes. It i characteristic behaviour of all living beings to produce tinually these great chemical and physical changes. The is not a mere test-tube collection of heterogeneous che substances, but must be looked upon as an active machine we can obtain no clear picture of its activities unless we upon it as acting dynamically. Unfortunately in a large nu of cases we are unable to investigate a plant physiolog without killing it, with the result that we are only seeing the static state, when we ought to be considering it fron dynamic standpoint. Such a state of affairs necessarily lea considerable gaps in our knowledge of the physiological acti of plants.

Metabolism is the main concern of plant physiology, and are two methods of approaching it. In the first place, the can be killed, and the various chemical compounds prese its cells extracted by appropriate means and their amount mated. From the results of such an analysis, an attempt can be made to deduce the chain of chemical reactions that takes in the plant when it is alive. A number of justifiable critic can, however, be made against such a procedure, for bet the living and dead plant there is a great gulf fixed. Secor an examination can be made of the external conditions u which a plant grows, such factors, for example, as light, perature, humidity of the atmosphere, the nature of the soil so forth, and by observing the effect of altering these diffe factors on the growth and well-being of the plant, and the in which they affect the rate of such processes as photosyntl and respiration, inferences can be drawn as to the nature of t processes.

A chemical analysis of a plant gives some idea of the var substances that are present, but it must be borne in mind the final result is a picture of a plant in the static state. E

when the analysis has been secured, it is exceedingly difficult to interpret the results correctly. It must be remembered that a number of different metabolic processes are taking place within the compass of a single plant cell. Take, for example, the unicellular green alga, Chlamydomonas. Here we have a complete living entity composed of one cell, which is capable of synthesizing carbohydrates and proteins and at the same time respires, while in the higher plants the matter is still further complicated by the question of translocation of the various substances elaborated in the course of active metabolism.

Although an analysis of a plant may yield valuable data as to the nature of the various compounds present, on account of their variety it becomes a matter of difficulty to say with complete confidence that these various substances are formed in a certain order or sequence. The problem of animal physiology in comparison with plant physiology is relatively simple on this particular score, for in higher animals at any rate, special organs are set apart for special purposes and these carry out their functions to the practical exclusion of all others. In the plant, on the other hand, the somatic tissues are far less correlated than in an animal and each individual cell has to carry out a variety of complex metabolic processes. With the limited methods of technique at present available, it becomes a matter of difficulty to ascertain the exact course of these various metabolic reactions.

Furthermore, it is not possible to judge of the importance and significance of a particular substance found by analysis to be present, by the actual amount that is discovered. Suppose, for example, that in the course of some chemical reaction taking place in a plant cell a substance A was being converted into B, and B in turn to C and C into another product D, thus:

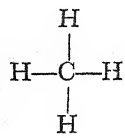
$$A \rightarrow B \rightarrow C \rightarrow D$$

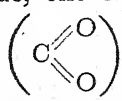
It is clear from such a series of transformations that it is impossible to assess the importance of any one of the substances involved in this reaction by the amount of any particular one found after death. The rates of reaction in each case may be different, thus A may be converted into B more rapidly say than C into D or B into C. Nor does it follow that substances isolated from the dead tissues of a plant are necessarily present when the plant was alive; they may be products of decomposition due to the method of killing the tissues.

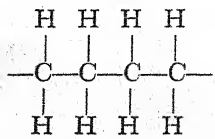
If an elementary chemical analysis be made of diff members of plants, such as wheat grains, beet leaf and p tubers dried at 100° C., the following average results wi obtained:

Material	*Carbon Per cent*	*Hydrogen Per cent*	*Oxygen Per cent*	*Nitrogen Per cent*	*A Per*
Wheat Grain ..	46·1	5·8	43·4	2·3	2
Potato	44·0	5·8	44·7	1·5	4
Beet Leaf	38·1	5·1	30·8	4·5	21

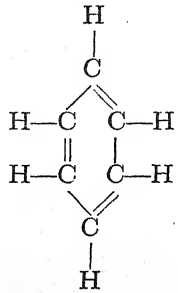
The richness of the carbon present should be noticed. presence of the element carbon is characteristic of all li organisms. Without it life as we know it would be quite possible. Carbon has the remarkable property of linking atoms together to an indefinite extent, and in this way is to build up highly complex compounds such as carbohydr and proteins. Since carbon functions as a tetravalent elen (except in certain special cases), each carbon atom is abl combine with four univalent atoms or two bivalent at For example, in the simplest of all the hydrocarbons, meth we have one carbon atom attached to four univalent hydr atoms:



while in carbon dioxide, one carbon atom is attached to divalent oxygen atoms  . In the case of the long-c carbon compounds that have been mentioned, each carbon a in the middle of the chain is capable of combining with hydrogen atoms:



Organic compounds possessing such a structure are termed open-chain or aliphatic compounds in contradistinction to the cyclic or aromatic organic derivatives in which a nucleus of six carbon atoms is present. The simplest of these aromatic compounds, and the one from which, theoretically, all the others are derived, is benzene:



The above analysis also shows that oxygen is present in high percentage. This is due to the presence of compounds, such as the carbohydrates, which possess a high percentage of oxygen in their molecules. Lastly it should be observed that nitrogen is always present.

With regard to the actual chemical entities that are to be found in plant tissues, we have proteins, amino-acids, amides, enzymes of different kinds and unknown constitution, organic acids, fats, carbohydrates, lipins, ethereal oils, alkaloids, glycosides, tannins, resins and a variety of pigments.

If a more detailed analysis be made of different plants in order to ascertain the precise amounts of some of the compounds mentioned above, the following average figures will be obtained for wheat grains, lupin seeds, potato tubers and lettuce leaf:

	Wheat Grains	*Lupin Seeds*	*Potato*	*Lettuce Leaf*
	Per cent	*Per cent*	*Per cent*	*Per cent*
Water	13·65	12·88	75·48	94·37
Dry weight	86·35	—	—	—
Nitrogenous substances ..	12·35	36·52	1·95	1·41
Fats	1·75	4·92	0·15	0·31
Fibre	2·53	14·04	0·75	0·75
Carbohydrates	67·91	27·60	20·69	2·19
Ash	1·81	4·04	0·98	1·03

In these sets of analyses the total ether extracts were taken to represent the percentage of fats. The nitrogen was estimated by the Kjeldahl method and the percentage of nitrogen thus obtained multiplied by the factor 6·25 to give the percentage of protein, while the fibre was that portion of tissue insoluble in dilute sulphuric acid and dilute sodium hydroxide solution.

The high percentage of water, except in the case of the lupin seeds and wheat grains, in these various tissues should be noticed. All active tissues contain over 75 per cent of water. In some of the algae, the percentage of water in the tissues may be as high as 98 per cent. The relatively high percentage of protein in the lupin seeds compared with the other examples may possibly be due to the symbiotic relations of these plants with nitrogen-fixing bacteria: a characteristic of the Leguminosae (see Chapter X).

As long ago as 1881, Reinke and Rodewald carried out a detailed analysis of the plasmodium of the myxomycete, *Fuligo septica*, to ascertain the composition of the protoplasm. The object of using a myxomycete was to eliminate the complications that would have been produced by the presence of cell walls, as the plasmodium of a myxomycete is to all intents and purposes a naked mass of protoplasm:

Water (at air-temperatures) 5 per cent. At 100° C. 75 per cent.
Dry Matter. (N.B. A large amount of calcium carbonate was found to be present.)

	Per cent
Protein (P free)	15
Protein (with P)	40
Amino-acids	1·5
Fats	12·0
Lecithin	0·3
Cholesterol	2·0
Carbohydrates	12·0
Resin	1·5
Salts	7·0*
Undetermined	8·7

A more detailed investigation of the plasmodium of the myxomycete *Fuligo varians* has been carried out by Lepeschkin† using more modern methods of chemical technique:

* I.e. other than $CaCO_3$.
† *Ber. deut. bot. Ges.*, 1923, **41**, 179.

(A) Water soluble organic substances chiefly contained in the vacuoles.

	Per cent
Monosaccharides	14·20
Proteins	2·20
Amino-acids, purine bases, etc.	24·30

(B) Insoluble organic substances principally contained in the ground mass of the protoplasm.

	Per cent
Nucleo-proteins	32·30
Free nucleic acids	2·50
Globulin	0·50
Lipo-proteins (plasmatin)	4·80
Neutral fats	6·80
Phytosterol	3·20
Phosphatides	1·30
Other organic matter (polysaccharides, pigments, resins, etc.)	3·50

(C) *Mineral matter* (about half extractable with water) 4·40

It cannot be said that either of these analyses cast any light on the nature of protoplasm.

Apart from reproduction, we have in all living organisms a co-ordinated series of chemical reactions taking place in a special substratum, the protoplasm, and to these co-ordinated chemical reactions we give the comprehensive name of *metabolism.* Metabolism has a credit and debit side; there is the *anabolic* or constructive side of metabolism whereby the living organism is able to build up different products, and the *catabolic* or breakdown side of metabolism, in the course of which compounds are broken down with simultaneous release of energy.

A number of suggestions have been put forward to account for the remarkable properties of protoplasm. One of the first of these was the so-called "Organization" Theory, which was widely held at one time. According to the organization theory, protoplasm was built up of small particles or corpuscles, which bore the same relation to protoplasm as say bricks do to a house. Charles Darwin termed these particles "genules," and held them responsible for the various manifestations of heredity. They were termed "pangenes" by de Vries, while Herbert Spencer spoke of them as "physiological units" and Weismann called them "biophores" or "determinants."

Protoplasm according to the organization theory was a complex

morphological entity. Unfortunately, such a view of the nature of protoplasm is not very helpful since it simply pushes the matter back a stage. The question at once arises: What is the exact nature of these particles composing protoplasm? Moreover, the organization theory overlooks the dynamic aspect of life: The power that life possesses of co-ordinating its various chemical and physical processes.

Another theory that was put forward to explain the nature of protoplasm was the so-called "Chemical" Theory. After the synthesis of urea by Liebig and Wöhler, it slowly came to be realized that there is no fundamental difference between the chemical compounds occurring in the organic and inorganic world, and that there is no special vital force necessary for the synthesis of organic compounds, and so gradually the idea was formulated that the proteins which are to be found in all living cells are the essential constituents of life. Thus, according to the chemical theory, the protein molecules in protoplasm were considered to be very labile and unstable in character. It was known that they possess a high molecular weight, and life was supposed to be carried on by the utilization of the energy released by the building up and breaking down of these giant protein molecules. According to Pflüger, for example, the process of respiration is due to the breaking down of these giant protein molecules and from the energy freed in the course of this process other protein molecules are built up once more.

In 1903 Verworn brought forward a "Biogene" or "Biophore" Theory for the structure of protoplasm. This theory is really an elaboration of the chemical theory described above. These biogenes were supposed to be giant protein molecules with elaborate side-chains, and it was further supposed that in the course of metabolism they broke down partially. The side-chains were considered to be the active parts of the molecules, and although the biogenes themselves were not supposed to be living they were thought to be the basis of life. Nevertheless, there are a number of difficulties to be contended with on Verworn's theory or on any purely chemical theory that attempts to explain the nature of protoplasm. In the first place, it must be remembered that the proteins from the chemical standpoint are relatively stable bodies, whereas, according to the various chemical theories we have had under consideration, they have been assumed to be very labile in nature. This objection was answered by saying

that in the living state they were labile! Death, i.e. death at senescence, should mean a change from lability to stability. At death, therefore, we should expect some profound chemical change with the liberation of considerable quantities of energy, and this is not the case.

The hypothesis of a labile chemical compound for the constitution of protoplasm does not assist in bridging over the gap between dead and living matter. In actual fact it merely shelves the problem. Modern views reject the idea of some special chemical substance being characteristic of life.

THE PHYSICO-CHEMICAL THEORY OF LIFE

According to the modern view of life and life processes, the activities of living organisms are not due to some one special chemical entity present in their cells, but that we have in all living matter a complex series of dynamical reactions taking place in a particular substratum, namely, protoplasm. Protoplasm has been termed by Thomas Huxley the "Physical Basis of Life." Life as we know it would be impossible in the absence of protoplasm, and it has been shown that the activities of protoplasm are due to its colloidal nature. Since protoplasm is built up by the living organism in the course of its metabolic activities, it must therefore be understood that protoplasm is itself the colloidal product of the chemical activities of living things.

In the living plant a number of substances in the cells are kept apart, for example, glycosides and the glycoside-splitting enzyme, emulsin. It is only when the tissues have been killed and ground together that these substances are able to come into contact and carry out their characteristic reactions. The cell-sap of Oxalis leaves is very acid, but is prevented from acting upon the chloroplasts by the vacuole membrane. When, however, the leaves are killed, either in steam or by chloroform, the green colour of the leaf changes to yellow, for the vacuolar membrane has now become permeable to the acid within the vacuole and this is able to diffuse through and attack the chloroplasts. Hofmeister looked upon the cell as a laboratory in which several chemical reactions were taking place at once, the various substances and the reactions being kept apart by means of membranes or partitions of some kind. Sir F. G. Hopkins* thought

* *Brit. Assoc. Repts.*, 1913, p. 652; *Nature*, 1913, 92, 213.

that "interplasmic" reactions might perhaps occur in which compounds elaborated by the protoplasm could be held responsible for the chemical changes, the reactions taking place between the molecular aggregates composing the protoplasm.

The living organism must be looked upon as a machine, but there are two marked differences between a plant or animal and a human-made machine. In the first place, a living organism, however lowly in the scale of life, is infinitely more complex than any man-made machine, and secondly, it is able to construct and repair itself by its own functional activities.

The present-day view that a living organism, whether it be plant or animal, is composed of a complex physico-chemical system of a special kind furnishes us with a working hypothesis on which to proceed. There are still vitalistic views held, mainly by the school of investigators headed by Sir J. C. Bose. It is true that in our present state of knowledge we cannot give a mechanical and chemical interpretation of all the phenomena which occur in living matter, and such phenomena must provisionally be termed vital. Meantime we may note that with each successive advance in physiological science, vital phenomena are more and more regarded as special examples of the operation of chemical and physical laws.

The importance of the proteins in living cells lies not so much in the fact that they are of any special chemical nature, but that they are colloids. It was shown by Emil Fischer that protein molecules, though large, are built up of amino-acids, and from the physiological standpoint, it is the colloidal properties of these large molecules that are our main concern. Fats, lipins, enzymes, carbohydrates are also present in the colloidal state in the living cell; these facts taken together lend considerable support to the theory of the colloidal nature of life.

CHAPTER II

COLLOIDS AND PROTOPLASM

SINCE protoplasm exhibits the properties of a colloidal solution, it will be necessary to consider the general properties of the colloidal state before proceeding to a discussion of protoplasm itself.

Colloids were first scientifically investigated by the English chemist and physicist Graham. Although colloidal solutions had been prepared before Graham published his results, it remained for him to place the subject on a scientific basis. Graham divided matter into two classes, *colloids* and *crystalloids*. Colloids were substances which in solution would not pass, or would only pass very slowly, through a parchment membrane, whereas crystalloids, on the other hand, diffused through very readily. The experiment whereby this separation was effected was termed *dialysis*. Graham conceived the idea that these colloids and crystalloids were two separate states or worlds of matter. This, however, is not the case, colloids being only a special state of matter, for practically all chemical substances can by suitable means be brought into the colloidal condition. Examples of crystalloids are urea, sodium chloride, and cane sugar, i.e. substances which can be readily obtained in crystal form. The term colloid is derived from the apparent similarity of many to glue (*kolla*, glue, *eidos*, form). A sharp differentiation between the two classes crystalloid and colloid cannot be maintained in practice, since different solutions are known which exhibit every gradation from total non-diffusibility through a membrane to rapid and complete diffusibility.

Substances like gold, silver, platinum and arsenious sulphide have all been produced in colloidal solution, and at the same time there are a large number of naturally occurring complexes, such as the proteins, tannins, starch, cellulose, etc., which are colloids. The essential properties of colloidal substances are not due to their chemical composition but to their physical state. It is possible to prepare a colloidal solution of a substance by one method and a crystalloidal solution (i.e. a solution that will rapidly diffuse through a parchment membrane) of the same substance by another. Further, a few naturally occurring

colloids, such as proteins have been obtained in the crystalline condition.

In essentials the difference between a true solution and colloidal solution depends upon the size of the particles in solution. Sodium chloride dissolved in water forms a true solution and consists of sodium and chlorine ions as well as undissociated molecules of the salt, whereas in colloidal solutions two distinct phases are present. When the relative sizes of the molecules of the solvent and molecules (or aggregates of molecules or particles) are not greatly different the solution behaves as a true solution. On the other hand, when the aggregates of molecules, or molecule of dissolved or suspended substance, is larger than the solvent, the solution exhibits definite colloidal properties. Haemoglobin is an example of a substance with a large molecule and of complex chemical constitution which exhibits colloidal properties in solution. The molecular weight here is about 16,500, and the diameter of the molecule $5 \cdot 5\ \mu\mu$ (where $\mu = 0 \cdot 001$ mm. $= 1 \times 10^{-3}$ mm. and $\mu\mu = 0 \cdot 000001$ mm. $= 1 \times 10^{-6}$ mm.). Haemoglobin, though in the molecular state, behaves in solution as if it were a colloid.

GENERAL PROPERTIES OF COLLOIDS AND COLLOIDAL SOLUTIONS

Colloidal particles have a low rate of diffusion and colloidal solutions exhibit a low osmotic pressure. Although the osmotic pressure of colloidal solutions is small, it is nevertheless real. It was at one time considered that the existence of osmotic pressure in colloidal solutions was due to the presence of contaminating electrolytes. It is important in investigations concerned with the measurement of osmotic pressure of colloidal solutions that no traces of electrolytes are present, as these will seriously affect the final results. Moore and Roaf* in a detailed examination of the osmotic pressure of gelatin solutions found that the osmotic pressure of a 10 per cent gelatin solution was 70 mm. of mercury at 26° C. The experiment was extended over a period of two months and the osmotic pressure remained constant at this value. The persistence of this pressure over this extended period of time shows that a true equilibrium was in existence, and that it was not merely a phenomenon due to slowly diffusing electrolytes.

* *Biochem. J.*, 1907, **2**, 34.

Metallic gold can be brought into colloidal solution by the reduction of gold salts with such reducing agents as hydrazine sulphate and hydroxylamine hydrochloride in the cold or formaldehyde in hot aqueous solution. Another method of preparing a colloidal solution of gold is to strike a direct current arc between two electrodes made of the metal beneath the surface of well-cooled water. This method can also be employed to prepare colloidal solutions of silver and platinum. Other methods of preparing colloidal solutions depend on the nature of the colloidal substance. These metallic colloidal solutions are termed *sols*.

These metallic sols appear clear and transparent to the naked eye and cannot be distinguished from ordinary true solutions by their external appearance. By means of a device known as the ultramicroscope, however, in which a beam of light is passed through the sol and viewed at right angles through a microscope, the particles of a colloidal solution can be observed in a state of active movement. The bright beam of light causes diffraction haloes round the colloidal particles, and viewed through the microscope, the haloes, being larger than the particles, can be observed. It has been ascertained that the light is often polarized. This polarization of light through the ultramicroscope was first demonstrated by Tyndall and is often spoken of as the *Tyndall phenomenon* or *effect*. Such particles observed under the ultramicroscope are termed *submicrons* and their dimensions have been variously estimated as lying between 1 to 100 $\mu\mu$, a considerable range in size. The volume of these particles can be obtained by counting the number in a given area, and then from the weight present in this area and the density of the particles the volume can be calculated. Particles smaller than submicrons are termed *amicrons* and include all the smaller molecules and ions.

It has already been said that colloidal solutions consist of two definite phases; the colloidal particles are termed the *disperse phase*, while the containing medium is termed the *dispersion medium* or is sometimes known as the *continuous medium*. The colloidal phase is occasionally spoken of as the *internal phase* while the liquid phase is termed the *external phase*.

There are various possibilities connected with these two phases, depending on whether they are gaseous, liquid or solid. Thus the following series are known:

Disperse Phase	*Continuous Medium*	
Gas	Liquid	— Foam
Liquid	Gas	— Mist
Liquid	Liquid	— Emulsion
Liquid	Solid	— Gelatin
Solid	Gas	— Smoke
Solid	Liquid	— Suspension
Solid	Solid	— Glass

Colloidal solutions which have solidified, taking on a jelly-like form, are termed *gels*.

Colloidal solutions have been classified in various ways. The best is that based on the disperse phase. Colloidal solutions in which the disperse phase consists of a suspension of solid particles in a solvent are termed *suspensoids*, whereas if the disperse phase consists of liquid particles, these are termed *emulsoids*. The distinction, however, is inexact and crude. It is on the relative size of the suspended particles of the dispersoid that the relative properties of the two depend.

The viscosity of suspensoid colloids is the same as that of the solvent; they have a negligible osmotic pressure, and the volume of their "solutions" is made up of the sum of those of suspensoid and solvent, so that no contraction takes place on adding them together. In emulsoids, on the other hand, the volume is less than the combined volumes of solvent and solute; the viscosity is greater than that of the solvent, and the solution shows a distinct osmotic pressure.

Certain colloids can be deprived of their continuous phase, and when subsequently suspended in the solvent, pass back into the colloidal state once more. Such colloids are termed *reversible colloids*. If they do not pass into colloidal solution again, they are called *irreversible* colloids. Agar and gelatin are examples of reversible colloids, and silicic acid an example of an irreversible colloid.

Suspensoid colloids carry an electric charge. In the great majority of cases suspensoids are negatively charged, but colloidal solutions of aluminium hydroxide, ferric hydroxide and certain basic dyes, such as methylene blue, carry a positive charge. In aqueous solution, electrolytes dissociate into an equal number of positive and negative ions, non-electrolytes, such as cane sugar, dissolve in water but do not dissociate and carry no electric charge. Colloidal particles, on the other hand, all carry the same charge. To ascertain the nature of the charge on the particles, a

direct electric current can be passed through the solution, when all the particles will migrate to one or other of the electrodes, depending of course on the nature of the charge. Thus when a colloidal solution with negatively charged particles has an electric current passed through it, all the particles will migrate to the anode; on the other hand, when the particles are positively charged, they will all migrate to the cathode. This process is spoken of as *cataphoresis*.

The origin of the electric charge on colloidal particles is not properly known, but it is possible that it may be due to ordinary ionic dissociation of either one large colloidal molecule (e.g. proteins) or of one of the molecules in a colloidal aggregate. Cataphoresis may be demonstrated in the living plant cell when the chloroplasts become affected.

It was discovered by Faraday that suspensoids can be precipitated by the addition of electrolytes. The first step in the precipitation appears to be the neutralization of the electric charge on the particles by the oppositely charged ions of the electrolyte, and the electrolyte is precipitated along with the colloid. The same effect can also be produced by the addition to one suspensoid solution of another carrying the opposite electric charge. The effect is mainly governed by the degree of valency of the active ion, thus trivalent ions are more effective than bivalent and bivalent more effective than monovalent ions. For example, it has been found that the aluminium ion which is trivalent is more active than bivalent calcium or barium, and calcium and barium more efficient than monovalent sodium in this respect. If the molar quantity of sodium ion necessary to precipitate a colloidal solution be x, then instead of the other metals, i.e. calcium and barium and aluminium being more effective in the ratio of $2x$ and $3x$, it has been discovered that they are more effective in the ratio of x^2 and x^3. The explanation lies in the fact that the aluminium ion carries three positive charges, and it is therefore more probable that one of the colloidal particles will meet one of the aluminium ions, rather than that one colloidal particle will come into contact simultaneously with two bivalent ions or three monovalent ions.

It can be readily demonstrated that the ions of the precipitating electrolyte are themselves carried down with the precipitated suspensoid. For example, if a sol of arsenious sulphide be precipitated with barium chloride, the solution shows an acid reaction. This is due to the formation of hydrochloric acid from

the chlorine ions of the barium chloride and the hydrogen ions of the water, since the barium ions have been removed on the precipitated suspensoid:

$$BaCl_2 \rightleftharpoons Ba^+ + 2Cl^-$$
$$2H_2O \rightleftharpoons 2H^+ + 2OH^-$$
$$2H^+ + 2Cl^- \rightleftharpoons 2HCl$$

The amount of precipitating electrolyte added to a colloidal solution will affect it in various ways. For example, the blood corpuscles of the dog-fish carry a negative charge and are precipitated by the addition of cerium chloride ($CeCl_3$). If a $0 \cdot 0008M$ (M = molecular weight) solution of cerium chloride be added to a sol of the blood corpuscles, the charge on the particles will be neutralized and they will suffer precipitation. If, on the other hand, an excess of cerium ions be added to the suspension, all the charges on all the corpuscles will be reversed and no precipitation will occur. Clay is precipitated at the mouth of rivers by the precipitating action of sodium chloride in the sea. In point of fact the precipitation of colloidal suspensions by electrolytes is a complex process and is not altogether solely due to the reaction between equal quantities of two electric charges. The precipitation appears to depend on the production of temporary inequality and irregular distribution of the electric charges. Thus if a quantity of electrolyte, which when added all at one time is capable of bringing about complete precipitation of a suspensoid, is added little by little it is ineffective.

In the study of living organisms we are more concerned with emulsoid colloids than suspensoids. In emulsoids we have droplets of one liquid suspended in another. Proteins, starch, gums, agar, cellulose and fats furnish examples of emulsoid colloids, while silicic acid is the only known example of an inorganic emulsoid.

Emulsoids, like suspensoids, carry an electric charge, but they are less sensitive to electrolytes than the former; in fact monovalent ions have little precipitating effect. This difference in sensitivity between these two types of colloidal solution can be demonstrated with arsenious sulphide (suspensoid) and egg albumin (emulsoid). Both arsenious sulphide and egg albumin are precipitated from colloidal solution by lanthanum salts. Lanthanum is a trivalent element and the lanthanum ion carries a triple charge. It has been shown that the concentration of

lanthanum salt necessary to precipitate the same amount of arsenious sulphide and egg albumin is in the ratio of $0 \cdot 00005M$ to $0 \cdot 002M$.

The charge on the liquid disperse phase of emulsoids is not constant, but is found to be dependent on the continuous medium. For example, the concentration of hydrogen and hydroxyl ions of the continuous medium materially affect the system. When an emulsoid is added to a suspensoid sol, the latter becomes very much less sensitive to the action of electrolytes. The sol is now said to be "protected." This phenomenon was discovered a number of years ago by Faraday, who found that the addition of "a little jelly" made his gold sols very much more stable to the action of electrolytes.

The Iso-electric Point.—It has already been shown that the electric charge on colloidal particles is neutralized by the addition of an ion with an opposite charge. Thus in acid solution egg albumin moves to the cathode, whilst in alkaline solution it migrates towards the anode. The condition in which the colloid is uncharged has been termed by Hardy the *iso-electric point.* The iso-electric point is of importance in connection with proteins (see Chapter X).

ADSORPTION

The particles present in a colloidal solution are in a very fine state of division, and as a result there is an enormous extension of surface compared with matter in the ordinary state. The surface of a body, either liquid or solid, is the seat of a special form of energy, termed *surface energy.* Now the surface energy of the particles of a colloidal solution depends upon their *specific surface,* which may be defined as the area of the surface of the particles divided by their volume. This surface energy is exhibited as surface tension forces. There is a special condition exhibited at the surface of liquids in that the molecules at the surface of a liquid are only affected by molecules in the liquid beneath, whilst molecules submerged in the liquid are completely surrounded by molecules like themselves. It follows, therefore, that the forces exerted on molecules situated at the surface of a liquid are from molecules in the layers beneath, and a definite film is formed at the surface of a liquid. This film tends to make itself as small as possible and consequently a high pressure is exerted on the molecules within the liquid. Water has the

highest surface tension of any known liquid except mercury. It has been found that in a drop of water 3μ in diameter, the force exerted on the water molecules within the drop is equal to one atmosphere.

Substances dissolved in water tend to lower the surface tension at the interface* between the solution and immiscible liquid or a solid. There is always an accumulation of free energy at these interfaces, and the amount of this energy is altered by the deposition of various substances at the interface.

According to the second law of Energetics, free energy is always tending to reach a minimum. It follows, therefore, that substances which tend to lower the surface tension will become concentrated at the interface because there will be a diminution of free energy brought about by this means. This local concentration of substances at the interface has an important bearing in connection with colloidal solutions, for any substance dissolved in a liquid in contact with another phase will be concentrated at the surface, for by so doing free energy will be decreased. This process is termed *adsorption*. The connection between surface tension and adsorption was first theoretically deduced by the American physicist Willard Gibbs in 1875 from the thermodynamical standpoint and by J. J. Thomson in 1888 from the dynamical aspect.

Gelatin and silicic acid in the gel state take up many dyes with rapidity. This is due to the adsorption of the dye by the colloid and not to any chemical reaction between the two, nor is it due to biparturition. Thus, when a solution of picric acid in water is mixed with ether, the acid is taken up in a definite ratio by the two liquids, and the concentration in each solution is doubled by doubling the amount of acid but the ratio remains the same. This is not the case in adsorption, for if the amount of substance to be adsorbed is doubled the actual amount adsorbed is not doubled.

Bayliss† using the dye Congo-red in the form of its sodium salt (the molecule of Congo-red behaves like a colloid in solution) was able to demonstrate that no chemical change is involved in adsorption. As an acid, Congo-red combines with bases, amongst others aluminium hydroxide. The true salts of aluminium and Congo-red are a deep red in colour and when aluminium hydroxide is added to the colloidal suspension of the sodium

* Where two surfaces are in contact, it is customary to speak of the common surface as the interface.

† *Proc. Roy. Soc.* (*Lond.*), 1911, 84B, 81, 229.

salt of the dye, the hydroxide is "dyed" with Congo-red by adsorption. It is only later, when the preparation has been left for some time at room temperatures that a secondary chemical change takes place with the alteration in the colour of the hydroxide from blue to the red of the true salt of the acid.

We have already seen that electrolytes are able to neutralize or reverse the charge on colloidal particles, depending of course on their concentration. The question arises as to the influence of electrolytes on the process of adsorption. It was shown by Bayliss* that if a strip of ordinary filter-paper were immersed in a solution of the sodium salt of Congo-red, it was deeply stained. On the other hand, if a strip of the so-called ashless paper were used, hardly any of the dye was taken up. On the addition, however, of the smallest amount of a neutral salt, such as sodium chloride, the purest paper became stained. The explanation lies in the fact that, like most chemical substances that are inert, paper has a negative charge in water. In a solution of the sodium salt of Congo-red in water, we have the dissociation of the molecule giving rise to positively charged sodium ions and the aggregated anions of the Congo-red with negative charges. The negatively charged paper will repel the negatively charged Congo-red anions, but will attract the positively charged sodium ions. If a sufficient excess of positive ions be present, these will first of all be adsorbed, causing a diminution or even abolition of the negative charge on the paper and the coloured anions can then be adsorbed.

It is a well-known fact that when water containing a colouring matter such as litmus or caramel is shaken up with finely divided charcoal, the latter on settling down carries the colouring matter with it, leaving the water practically colourless. Charcoal also has the power of taking up gases, especially those that are easily liquefied, such as sulphur dioxide and ammonia. There is no chemical reaction involved in any of these changes, these substances being adsorbed on the surface of the charcoal. The dyeing of fabrics and the staining of sections are all phenomena due to adsorption.

Temperature and Adsorption.—A rise in temperature tends to lower the rate of adsorption of substances, for adsorption is an equilibrium process. A substance is adsorbed until equilibrium is reached, and should the temperature be raised, the equilibrium

* *Biochem. J.*, 1906, **1**, 175.

point is also altered and in this case shifted back. Bayliss in his researches on Congo-red found that two curves were obtained by treating filter paper with the dye at 10° C. and 50° (Fig. 1). It will be seen that in the curve obtained for adsorption at 50° C., the equilibrium point was reached more rapidly than at 10°, but that the actual amount of dye adsorbed was less.

Dewar* found that in the case of gases adsorbed by charcoal, if he placed charcoal in his vacuum flasks, and then employed

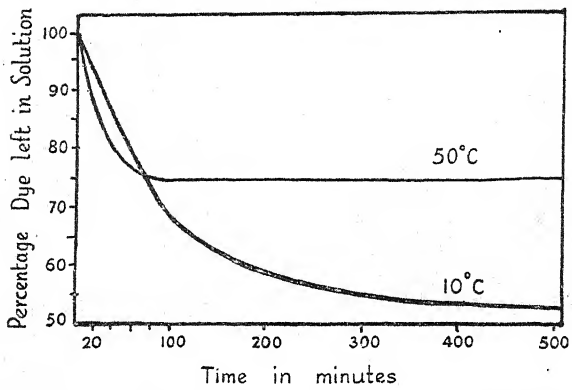


FIG. 1.—Curves showing adsorption of Congo-red by filter paper at 10° C. and 50° C. Notice that at the higher temperature the velocity is greater until the equilibrium position is reached. After Bayliss.

very low temperatures, adsorption was so complete that a very high vacuum could be obtained. The amount of a gas adsorbed is always higher the lower the temperature.

	Volume adsorbed at 0° *C.*	− 185° *C.*
Hydrogen	4·0 c.c.	135·0 c.c.
Helium	2·0 ,,	15·0 ,,
Nitrogen	15·0 ,,	155·0 ,,
Oxygen	18·0 ,,	230·0 ,,

One of the great advantages of this process of adsorption of gases by charcoal lies in its extreme rapidity; over 90 per cent of the total quantity adsorbed is taken up in the first few seconds.

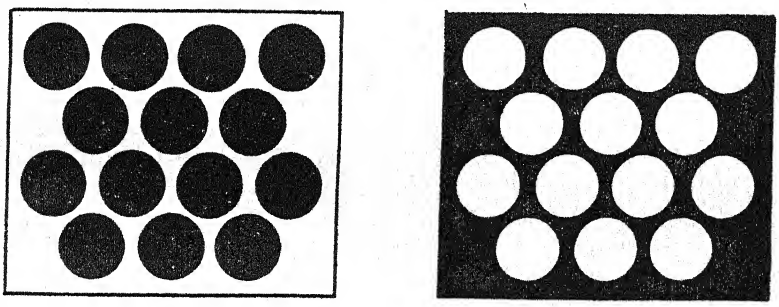
One of the best formulae to express the amount of substance that will be adsorbed in a given case is:

$$\frac{x}{m} = Ac^{\frac{1}{n}}$$

* *Proc. Roy. Inst. Gt. Bt.*, 1905, **18**, 177.

where x is the amount of substance adsorbed, m gives the total surface of adsorbing material, c is the final concentration of the solution and A and n are constants, which vary with the temperature.

Gels.—We have in agar and gelatin two typical examples of gels. The true nature of gels is still obscure; we can, however, obtain some idea of their nature by an examination of a gelatin gel which has been treated with formaldehyde and one that has not been so treated. In the case of a gelatin gel that has been



(A) (B)

FIG. 2.—Diagram to show the different phases of a colloidal system. A shows an ordinary hydrosol in which the black areas are supposed to depict the solid phase. B illustrates the structure of a hydrogel in which drops of water are embedded by the solid phase.

treated with the aldehyde, it is found that water is readily squeezed out, even gentle pressure with one's hands being sufficient to do this. On the other hand, with an untreated gel, a pressure of nearly 26 atmospheres is required before any water is removed.

To account for this it has been supposed that in the formaldehyde-treated gel we are dealing with a liquid continuous phase, with the solid particles embedded in it, with the result that when a slight pressure is applied the liquid is forced out (Fig. 2A), whilst in the case of the untreated gel, it is the solid phase that is continuous with the liquid embedded in it (Fig. 2B). Various intermediate stages are known between these two extremes and can be obtained by alteration of the liquid phase present.

PROTOPLASM

The nature of protoplasm has long given rise to much speculation among biologists. The word "protoplasm" was first coined

by Von Mohl in 1846 for the fluid, rather slimy substance always found in living cells. Von Mohl's investigations were concerned with plants, but the zoologist Dujardin many years before had named the substance present in the formanieran body, and in the body of lower animals generally, "sarcode." It remained for Cohn to realize that the protoplasm of the botanist and the sarcode of the zoologist were one and the same thing, and that this fluid slimy stuff was the basis of all life.

Early theories on the nature of protoplasm considered it to be a solid. The apparent lack of movement in a large number of animal cells and the frequent detection of structure suggested the existence of some solid substratum. It therefore came to be thought that protoplasm must be a contractile solid of complex nature which contained fluid in its interstices.

To this early idea of the nature of protoplasm can be traced the reticular and fibrillar hypothesis of protoplasmic structure. Thus Heitzmann claimed that protoplasm possesses a delicate reticular structure, whilst Flemming considered that a more or less filamentous structure is present. The "fixing" agents employed in the early days of cytology lent credit to these views, and cytologists failed to realize that they were in reality dealing with artifacts. It remained for Butschli in 1876 to show that protoplasm is essentially a liquid. From his experiments with various oil emulsions, Butschli arrived at the opinion that protoplasm possesses a foamy or alveolar structure. Hardy showed that the various structures described by cytologists were artificially produced by the "fixing" agents employed, and that different agents produced varying results depending on the time the agent was allowed to react, strength of agent and other factors.

The protoplasm of a cell consists of two parts, a fluid termed *cytoplasm* and a denser more viscous body, the *nucleus*. It is the nucleus that is probably the all-important part of the cell. The nucleus is spherical in shape, has no visible structure in the living condition, is more or less transparent and not very different in this respect from the surrounding cytoplasm, and therefore not easily observed in the living state. When fixed and stained, it shows on its outer surface a network of fine threads which is termed the *chromatin reticulum*. Certain darkly staining bodies, the *nucleoli*, are present within the nucleus. Their function is still obscure. It has been suggested that they supply chromatin to the

developing spireme when the nucleus divides (see below), but the evidence for this suggestion is very dubious.

The function of the nucleus is to govern and control the metabolic activities of the cell. No cell can carry out its vital activities in the absence of a nucleus. The nucleus divides, except among the very lowest organisms, by a complicated process termed *mitosis*. In the course of this process, a number of rod-shaped bodies, the *chromosomes*, are formed, each of which splits longitudinally; the daughter halves then separate into two groups and a new nucleus is formed from each group. The early phase of nuclear division is termed the *prophase*, when the chromosomes, in suitably fixed and stained material, can be seen as thread-like bodies. The nuclear membrane now disappears and a spindle-like structure arises in the centre of the cell; this may arise from a special body called the *centrosome*, which occurs with the nucleus in the cells of lower plants (e.g. algae and fungi) as well as in the cells of animals, but is absent in the higher plants. In the latter there is a good deal of controversy as to how the spindle does arise. In fact there is considerable doubt as to the exact nature of the spindle (see below). The chromosomes now become attached to the equator of the spindle and there accurately divide into two; the daughter halves passing to either pole of the spindle. Once arrived at the poles, the chromosomes condense together, and finally loosen out to form the reticula of the two daughter nuclei. In animals a new cell wall is formed by cleavage; a similar state of affairs is to be found among some of the lower plants. In plants generally, a cellulose wall is laid down across the equator of the spindle, which at the same time greatly increases in width. When the nucleus is not actively dividing, it is said to be in the *resting condition*. This is an unfortunate term as it is in the resting condition that the nucleus is physiologically most active.

Protoplasm is undoubtedly a colloidal system. The evidence for this view is overwhelming. The probabilities are that it is a polyphase system. It has been suggested that protoplasm is a gel, but in the so-called "protoplasmic streaming" that is to be seen in the cells of such a plant as Elodea, for example, we have strong evidence that in protoplasm we are dealing with a liquid system. The manner in which the cytoplasm, in the streaming movement to be observed in the cells of Elodea, passes round the corners of the cell can only be accounted for on the supposition that we are dealing with a sol and not a gel. Further, in the

plasmodium of a myxomycete, such as Badhamia, the protoplasmic mass can flow through a lump of cotton wool; and in this way any food particles present are removed. For the protoplasm of the organism to flow through cotton wool in this manner shows that once again we are dealing with a liquid system and not a gel.

Further evidence that protoplasm is a liquid comes from the micro-dissection work of Chambers and others. In this method the living cell is pierced with long glass needles drawn out to a fine point and usually bent at right angles to facilitate working. The needles are held in a special piece of apparatus known as the micro-manipulator of which there are a number of different types. By careful manipulation the needles are allowed to enter the cell, and the effect is observed under the microscope. Chambers* found that when the eggs of Fucus were pierced and the needle withdrawn there was no liquid on it. Presumably the fluid had flowed back into the egg, giving further proof of the liquid nature of cytoplasm.

Particles present in cytoplasm show *Brownian Movement.* This Brownian movement was first discovered in 1827 by the botanist Robert Brown, who showed that pollen grains and the spores of Equisetum exhibit a constant movement when suspended in water. Colloidal solutions also show this movement. "They go and come, stop, start again, mount, descend, remount again, without in the least tending towards immobility" (Perrin). It was at one time thought that this curious movement might be due to convection currents, electrical charges and other causes. Later investigations, especially those of Ramsay, have shown that this Brownian movement of particles in colloidal solution is due to the bombardment of the particles by the molecules of the liquid phase in which they are suspended. Observations with the ultramicroscope have established that the smaller the particles the more brisk is the movement, and the movement becomes more rapid with rise of temperature. In the case of a large particle, the chances are that the molecules of the suspending liquid would strike it with equal force on all sides, with the result that the blows would be counterbalanced and the particle would eventually sink under the influence of gravity. On the other hand, with a small particle the bombarding molecules (owing to the smallness of the size of the particle) would be unable to strike it equally

* *Amer. J. Physiol.*, 1917, 43, 1.

on all sides at once, a series of blows would therefore be delivered in different directions, and the particle would be kept in a state of active motion. This Brownian movement is the principal cause of maintaining the permanency of colloidal solutions.

Returning again to the question of protoplasm, it is obvious that cytoplasm could only demonstrate Brownian movement if it were a sol and not a gel.

The viscosity of cytoplasm has been a matter of considerable controversy. Cytoplasmic viscosity seems to be variable and to vary with different conditions of development. Thus there is a rapid increase in the viscosity of the cytoplasm of the eggs of Fucus towards the end of the ripening process, but at fertilization there is a return to the liquid condition with a fall in viscosity. Heilbrunn* has criticized the various methods that have been employed from time to time to measure the viscosity of cytoplasm, and considers that the micro-dissection method which has been much used for this purpose is by no means ideal and does not give accurate results. "For the measurement of viscosity, the micro-dissection method can at best give only indications of gross differences in viscosity, and even for these it is more or less uncertain." The entrance of the needle into the cell necessarily causes injury and this might possibly affect the viscosity of cytoplasm. Heilbrunn from his own experiments on the viscosity of the cytoplasm of the endodermal cells of *Vicia Faba* by observing the fall of starch grains in these cells and comparing the rate with the rate of fall of starch grains in water, considered that the viscosity of the cytoplasm was approximately eight times that of water. He also investigated the viscosity of the cytoplasm in the plasmodium of a myxomycete. In this method small iron particles were introduced into the plasmodium, which was then placed under the influence of a magnetic field from an electric magnet. The viscosity could be measured by the extent to which the particles turned under the influence of the magnetic field. In this case the results showed that the viscosity of the cytoplasm was from nine to eighteen times that of water. There can be little doubt that the viscosity of cytoplasm varies at different times in its life, but it is important to bear in mind that it is physical structure and not viscosity which determines whether a system shall be a sol or a gel. Hence viscosity measurements in themselves are not sufficient to prove this point.

* *Proc. Soc. Exp. Biol. Med.*, 1921, **19**, 87; *Biol. Bull.*, 1921, **41**, 318.

Bayliss,* for example, found that active Brownian movement was present in the cytoplasm of amoebae when observed under the ultra-microscope. If the amoebae were submitted to an electric shock the movement ceased and the cytoplasm coagulated. Bayliss showed that the force of the shock could be so regulated that only temporary coagulation of the cytoplasm occurred, and he then noticed that after a time lag the particles recovered their power of active oscillation. Price† has demonstrated that in the resting spores of Mucor the cytoplasm is in the state of a gel, but when the spores begin to germinate the cytoplasm becomes liquid.

Protoplasm shows the phenomenon of adsorption. It is capable of taking up certain dyes, for example, like any other colloid. It is true that it is difficult to stain protoplasm when it is in the living state, but this difficulty is due to the presence of a semi-permeable external membrane, which only becomes permeable when protoplasm is killed. But intra-vitam dyes are now known.

The filaments of the coenocyte Vaucheria occasionally become broken into separate pieces, and in these circumstances the protoplasm flows out, and in the presence of water becomes spherical, with a membrane surrounding it. Any naked mass of protoplasm develops such a surrounding film when it comes into contact with water, a result that is brought about by the adsorption of salts on the surface, the salts being obtained either from the surrounding water or from the interior of the protoplasmic mass. The membrane that is formed in such circumstances is apparently of gel nature.

Up to the present we have seen that cytoplasm is a complex emulsoid, and that at the same time it is a hydrosol, the continuous phase being water containing proteins, fats and other bodies in suspension as the disperse phase. Perhaps it would be better to speak of the continuous phase as being a watery one; it may be either pure water or a weak solution of proteins.

The Nucleus.—Within recent years a considerable amount of work has been carried out on the cell nucleus by Chambers and others using the micro-dissection method. A large amount of information is available with regard to the nucleus in animal tissues through micro-dissection studies, but unfortunately our information is comparatively meagre for plant tissues. The reason for this lack of knowledge is a purely mechanical difficulty. The presence of a resistant cellulose wall in plant cells does not allow

* *Proc. Roy. Soc.* (*Lond.*), 1920, **91B**, 196. † *Ann. Bot.*, 1914, **28**, 601.

of the easy entrance into plant cells of the delicate glass needles used in this type of work, for they are quickly fractured when they encounter such an obstacle.

Chambers* was able to remove the nucleus intact from the egg cells of the star-fish when he found that it either burst or set into a coagulated body which could be cut in pieces. He also ascertained that if the nuclear membrane were torn, the surrounding cytoplasm liquified in a few minutes. When, however, the rupture of the nuclear membrane was carried out with great care, liquefaction was found to set in slowly and was quickly limited in extent by the formation of a film at the boundary between disintegrating and healthy cytoplasm. When this film was in turn ruptured then a second film was often formed.

Chambers showed that in the nuclei of egg-cells of the star-fish a chromatin reticulum could often be discerned, whereas in the case of the somatic cells the appearance of a granular network was more frequent. In the grasshopper (*Dissosteira Carolina*), it was discovered that on injury the nuclei of the developing spermatocyte form more or less granular filaments. Loops of the filamentous tangle could be caught on the end of the micro-dissection needle and pulled out into an attenuated strand. When they were released they slowly contracted. Finally, it was found that the filaments developed into short rod-like bodies, which Chambers considered to be chromosomes precociously formed. If these chromosomes were left to themselves, they clumped together into an irregular, glutinous mass. When the nuclei were removed in Ringer's solution (see p. 102) the nuclear matrix gradually absorbed water, became swollen and then disappeared and the chromosomes were left free, and then they too gradually swelled up and passed into solution.

According to Chambers, three stages can be distinguished in the development of the growing spermatocyte depending on the way the prophase nuclei react to mechanical injury. In the first stage, nuclei respond to injury by revealing delicate granular filaments which shorten and thicken into homogeneous appearing rodlets. In the second stage, injury produces the immediate appearance of early prophase chromosomes which then precociously resolve themselves into what appear to be typical metaphase chromosomes. In the third stage, prophase chromosomes are already discernible in the uninjured nuclei and injury accelerates

* *J. Gen. Physiol.*, 1921, 4, 41.

their transformation into compact, metaphase chromosomes. It would seem that in a nucleus which is preparing for mitosis, mechanical injury with the micro-dissection needle hastens in some unknown way the normal routine of chromosome formation.

The mitotic spindle or achromatic figure has also been investigated by micro-dissection methods. In fixed and stained preparation, the achromatic figure shows up as a spindle-shaped structure composed of a number of fine threads, the spindle fibres. The exact nature of the spindle is still a matter of doubt. In the sand-dollar egg, the spindle apparently changes in viscosity during division. The viscosity first rises to a maximum and then falls. Chambers and Sands* have been able to see the spindle outlined against the surrounding granular cytoplasm in the pollen-mother cells of the Spiderwort (*Tradescantia virginica*). The spindle area here forms a hyaline jelly-like mass, less solid than the surrounding cytoplasm, and can be seen to be distinctly separated from it. Chambers and Sands were unable to find any evidence of the presence of fibres in the spindle in the living state.

The metazoan nucleus has been described by Chambers as being fluid in nature, showing no visible structure, possessing a nuclear membrane and containing one or more nucleoli. Plant nuclei, on the other hand, show a wide range of variation. Thus in Symphoricarpus and Spirogyra there is apparent homogeneity, whilst in Elodea the nucleus shows a fine-grained heterogeneity, and in Tradescantia a coarsely mottled appearance.

The nucleus in Spirogyra takes the form of a smooth, transparent sphere when the cytoplasm is stripped away. When punctured with the needle the contents are ejected with some violence. The liquid portion is quickly dispersed and the solid part then becomes visible. When the nucleus of Tradescantia is punctured in this way, the solid portion shows an irregular, folded mass which corresponds with the more highly refractive portion of the normal uninjured nucleus, whereas the fine-grained nucleus of Elodea when punctured ejects its contents as small irregular lumps.

There is a considerable conflict of evidence with regard to the origin of the nuclear membrane. Some have considered it to be of cytoplasmic origin. Scarth† considers that two membranes are present, one on the inner face of the cytoplasmic envelope surrounding the nucleus, and the second on the surface of the

* *J. Gen. Physiol.*, 1923, **5**, 815.

† *Protoplasma*, 1927, **2**, 189.

nucleus itself, and that the two are separated by a hyaline solution. Thus, according to Scarth, the nuclear membrane is partly cytoplasmic and partly nuclear in origin.

According to Chambers, the isolated chromosomes of plants and animals are similar in their consistency, but plant chromosomes are more resistant to injury, whereas animal chromosomes soon disintegrate after removal from the nucleus.

It is obvious from what has already been said, about both the nucleus and cytoplasm, that colloids must play an enormously important part in the life and behaviour of protoplasm, since protoplasm is itself a colloidal system. The characteristic of adsorption which colloids possess gives to protoplasm the power of taking up a large range of different substances, both electrolytes and non-electrolytes. If any of these substances lower the surface tension of the continuous phase of protoplasm, they will be adsorbed on the particles in suspension. Thus, by adsorption, they will be thrown out of solution and can no longer influence, say, the osmotic pressure of a cell, nor will they be able to diffuse from cell to cell.

Another important aspect of adsorption must be considered in connection with protoplasm. By adsorption, substances in the cell will be brought into more intimate contact with the colloidal aggregations present, such as enzymes, proteins and so forth, and chemical processes, which under laboratory conditions can only be brought about by high temperatures and the presence of strong acids or alkalis, take place at ordinary temperatures in the living plant. Further, since these substances are adsorbed on the surface of the particles they will be in a state of high concentration, and Guldberg and Waage's *Law of Mass Action*, which states that the amount of a chemical reaction is proportional to the active mass of each of the chemical substances reacting, active mass being defined as the molecular concentration of the reacting substance, will come into play. The importance of this law is that chemical activity of a substance is not proportional to the quantity present, but to its concentration, i.e. the amount in unit volume of the reaction mixture.

Chemical reactions take place very much more readily and smoothly on surfaces than they do elsewhere. This is readily shown by such a simple reaction as that between hydrogen and oxygen to form water. If this reaction be allowed to proceed in a glass vessel, then the temperature must be raised to 600° C. before

combination will occur. If a silver vessel be used instead of glass, a temperature of 100° C. is needed, whilst in a platinum vessel the temperature is still lower for explosive combination to take place, namely, 30° C., and lastly with "spongy platinum," a temperature of only 10° C. is required. The examples cited illustrate the importance of surface activity. Spongy platinum possesses the greatest extension of surface, and combination of hydrogen and oxygen takes place without any preliminary heating. Thus it will be seen that the colloidal aggregations in the living cell, presenting as they do a wide surface area, allow of chemical reactions to proceed in the living organism at ordinary temperatures, which under laboratory conditions can only be brought about at high temperatures and prolonged boiling.

It has already been stated that the metals gold, silver and platinum have all been obtained in colloidal solution. Bredig has shown that these metals in a fine state of division are powerful catalysts, and are able to catalyse a number of chemical reactions, just as the organic catalysts isolated from the cell, the enzymes, are able to catalyse a large variety of reactions. Spongy platinum is able to decompose hydrogen peroxide into water and oxygen, just as the enzyme catalase decomposes the peroxide. These various catalytic reactions are brought about by the wide extension of surface presented by these metals in a fine state of division. Bredig has termed them "inorganic enzymes." They certainly show parallels in their behaviour to enzymes, for example they are inhibited just as enzymes are inhibited by such toxic substances as hydrogen cyanide.

It will now be seen how protoplasm by adsorption is able to bring about various chemical reactions that could not otherwise take place under the conditions of the living cell. For example, by alteration in the size of the particles present, the total surface of the particles is necessarily increased or decreased, and this will result in changes in the rate and amount of adsorption, and consequently in the velocity of the chemical changes taking place on the surface of the particles. The rate of adsorption is also dependent on such factors as temperature and electrical charges on the particles. Variations in these different factors will react on the rates of the chemical reactions taking place in the cell, or in other words bring about variations in the metabolic rate. Should the surface tension of protoplasm be disturbed in any way, the concentration of some of the active substances present will also

suffer alteration, and this will be followed by changes in the rate of adsorption. In this way protoplasm is able to control its metabolic activities.

A further illustration of the importance of surface activities in physiological processes is given by Warburg's* experiments with the eggs of sea-urchins. The eggs were first immersed in solutions of various dyes, such as neutral red, which are able to penetrate the protoplasm, but do not kill it. The eggs after staining were placed in a 1 per cent solution of sodium hydroxide, when no change in the colour of the dye occurred. The hydroxide had therefore not penetrated the eggs. It was discovered, however, that the hydroxide had a powerful influence on the membrane surrounding the eggs, for the rate of respiration rose to 100 per cent of its former value. This rapid rise in the respiration rate must have been due to some specific action of the hydroxide on the outer membranous surface of the eggs, since no penetration had taken place. On the other hand, when ammonia was used in place of sodium hydroxide, rapid penetration was found to take place, the colour of the neutral red turned to yellow; but in spite of entrance of the alkali, the respiration rate only rose by 10 per cent.

According to V. H. Blackman and Paine,† the bending of the pulvinus of the sensitive plant, *Mimosa pudica*, is due to adsorption changes in the protoplasm of the cells of the pulvinus so that normal turgor cannot be maintained. The sensitivity of the pulvinus is brought about by variations in the turgor of the cells; the cells on the lower side of the pulvinus lose their turgor on stimulation, and the pulvinus bends over. It was at one time thought that this bending was due to osmotic pressure changes, the cell walls were supposed to become suddenly permeable and the osmotic substances were able to pass out of the cells, and in this way a collapse of the cells was brought about. Blackman and Paine cut the pulvinus under water and found that it was still sensitive after ten or a dozen stimulations. Moreover, if the theory that collapse is dependent on changes in osmotic pressure be correct, there must have been a large store of material present for the cells of the pulvinus to build fresh supplies so as to affect the osmotic pressure after each stimulation. It was also ascertained by Blackman and Paine that when the pulvinus was cut under water the amount of salts that leaked out was small.

* *Zs. physiol. Chem.*, 1910, **66**, 305.

† *Ann. Bot.*, 1918, **32**, 69.

Protoplasm is an unstable complex. It is affected by slight changes in the environment, markedly by temperature and even by shaking. For example, if a thread of Spirogyra be lifted up with a needle a visible alteration in the protoplasm can be observed at the point where the needle was applied. Protoplasm is also affected by the concentration of salts in the surrounding medium, humidity of the atmosphere, amount of radiation and so forth. The above considerations are true of colloids in general; they are also affected by alterations in temperature, concentration of salts, etc., solutions of certain enzymes when shaken are often made inactive, whilst protein solutions are sensitive to heat and the addition of electrolytes.

Protoplasm must also be considered in relation to the time factor. Any living cell of a higher plant, considered as an individual, grows, develops and then dies. There is a more or less restricted time during which it exists. This is not true of all cells; among unicellular organisms, after attaining a certain size, the cell divides and this process is continued indefinitely and the protoplasm is here potentially immortal.

When protoplasm is poisoned with weak solutions of toxic substances, the effect increases with time. The matter is not entirely one of accumulation and is not on the same footing as when strong solutions of toxic bodies are used. In its relation with time and temperature, protoplasm shows an analogy with proteins. We know that protoplasm is killed by heat, but it cannot be said with any degree of definiteness that death occurs at a certain temperature. Both time and temperature must be intimately related in this connection. It is a well-known fact that high temperatures bring about rapid death, while low temperatures do not. The probabilities are that the cell dies in parts.

There are certain arbitrary tests which can be used to show that a cell is dead: (1) the cell loses its semi-permeability and (2) dyes and stains are taken up. Lepeschkin* has studied the connection between time and temperature in their relation to the death of a cell, in *Beta vulgaris* and *Tradescantia virginica*. An arbitrary colour standard was used (0·00001 per cent solution of fuchsin) to denote when the death point had been reached, and this was matched against the colour which diffused out of the cells of these two plants. The time taken for the pigments in the cells to diffuse out and match the colour standard was recorded.

* *Ber. deut. bot. Ges.*, 1912, **30**, 703.

Lepeschkin worked with slices of tissue of a *given volume* and so exposed a *given surface* for a *given time* to a *given volume* of water. The following results were obtained for *B. vulgaris:*

Temperature (0° C.) ..	80°	70°	64°	50°	45°
Time (in minutes)	0·1	0·7	1·6	2·3	30

A simple equation was also obtained to express these results:

$$T = a - b \log z$$

where T is the time for death to take place, a and b are constants and z is the heating period. With this expression it is possible to calculate the time it should take a cell to die at any given temperature. Lepeschkin has calculated that at a temperature of 20° C. the cells of *T. virginica* should take thirty-three days to die, while for *B. vulgaris* at this temperature death should take place in thirty-one hours, whereas at 0° C. the cells of *T. virginica* should die in three years and *B. vulgaris* in eleven days. These results though apparently nonsensical when taken by themselves have a significance which will be discussed below.

The heat coagulation of proteins has been investigated by Buglia, who has shown that there is no absolute temperature at which coagulation takes place; it is largely a question of time. Coagulation of proteins is more rapid than coagulation of protoplasm. Buglia has shown that theoretically blood-serum, which coagulates slowly, should clot in forty-two years. Coagulation is always going forward in the living cell, but so slowly at the ordinary temperature that the process is balanced. At higher temperatures the action becomes more rapid and the balancing process cannot maintain equilibrium with the coagulating one. Lepeschkin's results given above take no account of this balancing process. The maximum temperature at which an organism can live is such that the balancing and coagulating reactions in the cell are just in equilibrium.

The previous treatment of matter often leaves some more or less permanent mark upon it. In the case of a piece of iron which has been magnetized by an electric current, it has been shown that if the current be decreased by small steps to zero there is no corresponding decrease in the magnetism of the iron bar; and when the current has been lowered to zero, the iron still retains a considerable amount of magnetism. This lagging phenomenon is termed *hysteresis*. It has been shown by Thoday and others that

the previous history and treatment of a plant has a very considerable influence on its after behaviour. The phenomenon of hysteresis is well shown by colloids. Van Bemmelen, for example, working with a silicic acid gel, showed that the amount of water taken up from the atmosphere by the acid at different temperatures increases with the vapour pressure (Fig. 3). When the moisture content was reduced, the second curve did not follow the course of the first. At a vapour pressure of 6·3 the water content in both cases was different (Fig. 3).

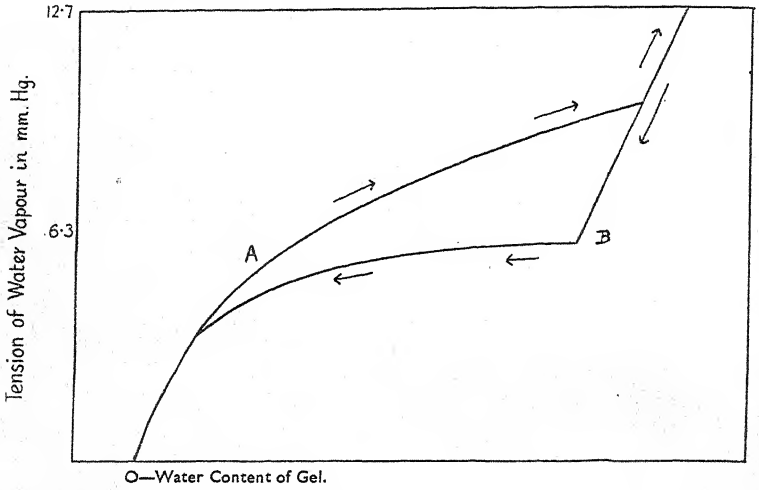


FIG. 3.—Graphical representation of the behaviour of a silicic acid gel in equilibrium with different tensions of water vapour. Curve A shows the behaviour of the gel when exposed to increasing tensions of water vapour and curve B when the gel is exposed to decreasing tensions. (After van Bemmelen. From Bayliss, *Principles of General Physiology*.)

Two marked characteristics of protoplasm are growth and differentiation. From the physico-chemical standpoint it is necessary to consider whether protoplasm looked upon in the light of a complex colloidal substratum, in which a series of co-ordinated chemical reactions take place, helps very far to a better understanding of these two phenomena. It cannot be said that it does. Both growth and differentiation are highly complex phenomena in so far as they depend on metabolism. It is not the function of science to answer the question *why* does a certain thing happen, but *how* does it happen; and the statements that protoplasm is a complex emulsoid colloid, or that it is a

polyphase colloidal system, do not take us very far on the road to understanding growth and differentiation of the living organism.

IMBIBITION

In a typical plant cell, such as a mesophyll cell of a leaf, we have first the outer dead cellulose wall, and within a thin lining of cytoplasm containing chloroplasts, a cell vacuole containing a watery fluid, the *cell-sap*, and finally the nucleus which presides over and governs the physiological activities of the cell. The primary physiological relations of the cell which we have to consider are the water relations of its walls. The cellulose wall is colloidal in nature and in this case is a gel. This membrane absorbs water; and this absorption of water can take place whether the cell be alive or dead, a process to which the name *imbibition* has been given. Imbibition is the power possessed by a gel of taking up relatively large amounts of water without actually forming a liquid solution. This water of imbibition can be given up again and the process can be repeated indefinitely. At one period it was considered that imbibition was due to the structure of the cell walls; it is now known to be a phenomenon associated with gels. The swelling of starch, aleurone grains and other substances is due to imbibition. The physical nature of imbibition is not understood. When such gels as gelatin, agar, etc., imbibe water and swell, the complexity of the process is shown by the fact that the increase in volume of these bodies is less than the amount of water taken up. This can be readily demonstrated by placing some gelatin or split peas in a flask filled with water and attaching a cork fitted with a long glass tube. The initial level of the water is marked on the tube. It will be found that the water level will at first show an increase, but as imbibition continues this initial rise will be followed by a fall.

The stipe of Laminaria is a favourite material for imbibition experiments. Reinke found that with cut slices of Laminaria stipe, the amount of pressure required to force out water of imbibition varied with the volume of water taken up:

Pressure in Atmospheres	*Percentage Increase in Volume*
1·0	330
3·2	205
7·2	97
21·0	35
41·0	16

It will be seen that the water is less and less firmly held as the maximum point of imbibition is reached. Reinke further showed that if the stipe were allowed to imbibe to its fullest extent and then exposed to the air, successive decreases took place in the amount of water present, and that the rate of drying fell away with loss of water. This was thought to be due to a fall in the vapour pressure, but in reality the main effect is due to the diminution in surface area, the evaporation from the smaller surface resulting in a fall in water loss.

When substances like agar and gelatin imbibe water, a certain amount of work is done in the subsequent swelling that takes place, and results in a considerable transfer of energy. The pressures exerted in this way are high and imbibition in plants has been put to such practical uses as the breaking up of rocks by pouring water upon wooden wedges driven into them, and the pulling apart of the bones of the skull for certain anatomical purposes. Heat is given out in the course of imbibition. With gelatin, the heat of imbibition is 6·0 calories per gram, whilst with starch and gum-arabic, 6·6 and 9·8 calories per gram are evolved respectively. The reason for this production of heat is not known, but the greatest amount of heat is evolved during the taking up of the first quantity of water.

Imbibition is markedly affected by the presence of acids, alkalis and electrolytes. In the case of electrolytes an increase or decrease of imbibition may result, the so-called Hofmeister's series being obtained.

Imbibition of Seeds.—The imbibition of seeds is a process not solely concerned with swelling. In the first stages of germination, water is taken up by imbibition alone, and it is only later that a cell vacuole is formed and osmotic pressure comes into play.

Temperature plays an important part in the imbibition of water by seeds, and in some experiments with red clover the following values were obtained:

Temperature	6 *Hours* *Per cent*	12 *Hours* *Per cent*	24 *Hours* *Per cent*	48 *Hours* *Per cent*
0° C.	60	89	107	115
10° C.	68	93	100	117
15° C.	100	113	111	116
35° C.	118	120	120	117

It will be seen that the total amount of water taken up is the same in all cases after 48 hours, but that at the other recorded times, temperature affects the rate of uptake.

IMBIBITION MECHANISM IN PLANTS

Many plants possess special mechanisms which depend on the dead tissues present in some part of their somatic organization taking up water by imbibition. Cases in point are the opening of fern sporangia and the capsules of mosses. The dehiscence mechanism in each of these cases depends on the alternate drying out and taking up of water by special tissues. Anastatica, the so-called Rose of Jericho, is another case in point. This plant grows under desert conditions in Palestine. It possesses a long tap-root which keeps it firmly anchored to the soil and in the dry seasons of the year the small aerial branches bend over and protect the fruit bodies. In the wet season, however, these branches take up water by imbibition and straighten out, and the fruit heads are exposed and can be distributed. In Erodium, the fruit has a long awn which is used in driving the seed into the ground. Under dry conditions the awn is twisted, and only straightens out when moist. The awn, by alternate contraction and expansion of its thickened tissues through imbibition of water, is able to drive the seed into the ground. It might be thought that each time the seed is driven into the ground in one direction by one twist, it would be lifted out again by a twist in the opposite direction. This is prevented by the presence of hairs on the seed coat which maintain the seed in the ground.

In the taking up of water by meristematic cells of plants imbibition at first plays a greater part than osmotic pressure owing to the absence of cell vacuoles.

CHAPTER III

OSMOTIC PRESSURE AND THE WATER RELATIONS OF THE PLANT

Osmosis is the term applied to the passage of liquids through a membrane. The existence of osmosis was first demonstrated in 1748 by the Abbé Nollet, who used a specially prepared pig's bladder filled with alcohol and fitted with a glass tube, and found that when the bladder was immersed in water, the alcohol was forced up the glass tube, and if the tube were closed in any way the bladder frequently burst. Thus water from outside had passed across the membrane of the bladder into the alcohol within.

It was shown nearly one hundred years later (1827–48) by Dutrochet and Vierordt, that if a salt solution were separated from water by an animal membrane, water from outside passed into the salt solution across the membrane more rapidly than salt diffused out into the water. The level of the solution within the bladder therefore rose and a hydrostatic pressure was established; and since this pressure was produced by osmosis, it was termed *osmotic pressure*. Dutrochet and Vierordt made a number of quantitative determinations on the osmotic pressure of different solutions, but their results are merely of historical interest owing to the crudeness of their technique. Maurice Traube was the first investigator to grasp the physiological importance of osmotic pressure in the case of plant cells, and it was largely due to his labours that the way was paved for later research. Traube in his investigations employed a number of different kinds of membranes, including one of copper ferrocyanide. The method of experimentation here was also very crude. A glass rod with a drop of copper sulphate at the tip was immersed in a solution of potassium ferrocyanide, a "cell" was formed in this way at the end of the rod, and using this fragile arrangement Traube was able to carry out a surprising number of important experiments. The membrane of copper ferrocyanide was found to allow the passage of some substances into the cell and not of others. For example, the impermeability of a membrane of copper ferrocyanide for certain dissolved salts may be illustrated by the following experiment. A glass tube fitted with a rubber tube and

clip at one end, and open at the other, is partly filled by suction with an aqueous solution of copper acetate (copper sulphate cannot be used here for obvious reasons) and ammonium sulphate; and the open end, in which the surface of the liquid has been made parallel by careful adjustment, is then cautiously dipped into an aqueous solution of potassium ferrocyanide containing a little barium chloride, and the tube supported in that position. A thin membrane of copper ferrocyanide forms across the lower end of the tube, and it will be found that even after standing for some hours there is no white precipitate of barium sulphate in the lower solution, showing that the membrane is impermeable to ammonium sulphate.

A membrane which allows of the passage of only one of the components of a binary system is termed a *semi-permeable membrane*. The membranes of pigs' bladder used by Dutrochet and Vierordt were permeable to both solvent and solute, but in proportion as the membranes employed by Traube were less permeable to the solute, so the observed hydrostatic pressure or osmotic pressure attained a maximum value.

Not much advance was made with this subject, as the semi-permeable membranes prepared by Traube were far too fragile to suffer rough handling or high pressures. In 1877 the German botanist Pfeffer hit on the brilliant idea of precipitating these membranes in the wall of a porous pot. He, like Traube, was convinced of the importance of osmotic pressure in the water relations of the plant cell, and having prepared these membranes which could be submitted to high pressures, he was able to study the matter from a purely physical aspect.

To prepare these porous pots with a lining of copper ferrocyanide, the pot is first well washed, soaked in water for some time and then nearly filled with a solution of copper sulphate (2·5 grams per litre), dipped to the neck in a solution of potassium ferrocyanide (2·1 grams per litre) and allowed to stand for several hours. The salts diffuse through the walls of the porous pot and at their junction form a membrane of copper ferrocyanide, which, since it is impermeable for the salts from which it has been formed, remains quite thin, and yet possesses strength to resist high pressures owing to its being supported by the walls of the porous pot. The "cell" is now well washed and filled with a strong solution of cane sugar, and closed with a well-fitting rubber cork through which an open glass tube passes (Fig. 4).

If such a cell be placed in water, the water passes in and the pressure inside gradually increases (shown by the rise in the level of solution in the glass tube), and finally attains a point at which it remains constant for days. A maximum pressure is thus attained and represents the osmotic pressure of this particular solution of cane sugar. In other words, the excess of pressure which must exist within the cell so as to prevent more water flowing in through the semi-permeable membrane, is the osmotic pressure of that solution.

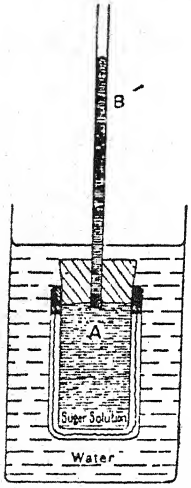


FIG 4.

When accurate measurements have to be made of osmotic pressure it is better to close the cell with a cork carrying a closed manometer containing a definite volume of air over mercury. In this way dilution of the solution by the entry of a large volume of water is prevented. Pfeffer used this arrangement in his original experiments.

Pfeffer was able to show that the osmotic pressure of a solution is proportional to the concentration, and he was also able to show that at constant concentration the osmotic pressure increases with temperature; but he did not discover the relationship that exists between osmotic pressure, temperature and volume, and it remained for the Dutchman, Van't Hoff, to correlate the whole matter. At the same time Pfeffer ascertained that colloids possess a low osmotic pressure, although the actual values he obtained were by no means accurate, as his colloidal solutions were contaminated with electrolytes.

Pfeffer's osmotic pressure determinations were made in 1877, and the degree of accuracy attained by him was not improved upon for nearly thirty years, when in 1903 the American investigators, Morse and Frazer, commenced their extensive and careful measurements, and a year later (1904) Berkeley and Hartly carried out similar determinations in this country.

In 1888 de Vries introduced his plasmolytic method of measuring osmotic pressure. The method is best employed with cells containing a coloured sap. Thin slices of the tissue are placed in different concentrations of a solution of, say, cane sugar. The concentration of solution which just causes the cyto-

plasm to be withdrawn from the cell wall is observed: the so-called "limiting plasmolysis" (Fig. 5). If the strength of the solution causing limiting plasmolysis be known, the osmotic pressure of the cells can be calculated. Solutions which have the same osmotic pressure are said to be *isotonic* or *isosmotic*.

It was shown by de Vries that gram-molecular solutions, i.e. equimolecular solutions, possess the same osmotic pressure, and using his plasmolytic method of determining osmotic pressure he was able to settle the molecular weight of the sugar raffinose. At

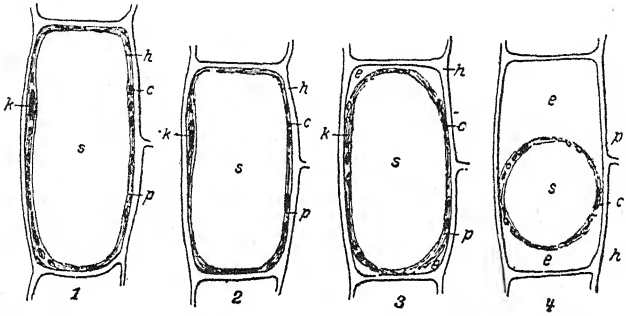


FIG. 5.—Various stages in plasmolysis. k = nucleus, p = cytoplasm, s = vacuole. (3) shows "limiting plasmolysis." (After de Vries.)

that time three rival formulae held the field, $C_{12}H_{22}O_{11}3H_2O$, $C_{18}H_{32}O_{16}5H_2O$, and $C_{36}H_{64}O_{32}10H_2O$ and the order of molecular weights for these three possible formulae is 396, 594 and 1,188. The concentration of raffinose necessary to bring about limiting plasmolysis in the cells of red beet was compared with the concentration of cane sugar required to bring about the same degree of plasmolysis. It was found that a 5·96 per cent solution of raffinose was isotonic with a 3·42 per cent solution of cane sugar. Since the molecular weight of cane sugar is 342, that of raffinose must be:

$$\frac{3 \cdot 42}{342} = \frac{5 \cdot 96}{M}$$

where M represents the molecular weight of raffinose. From this equation $M = 596$, which gives the formula $C_{18}H_{32}O_{16}5H_2O$ for the sugar. This value has since been confirmed by independent chemical means.

It is clear that if equimolecular solutions have the same osmotic pressure, this only applies to non-ionizable substances like cane sugar, for this statement can only be true if the substances not only remain equimolecular in solution, but of equal particle number, whatever may be the nature of these particles. Thus a solution of potassium nitrate may be equimolecular with one of cane sugar, but the osmotic pressure of the potassium nitrate will not be the same as that of the cane sugar solution, on account of the dissociation of the electrolyte into potassium and nitrate ions. If complete ionization were to take place in solution, then the osmotic pressure of the potassium nitrate solution should be double that of the solution of cane sugar. Actually this is not the case, the osmotic pressure is never quite double, and similarly divalent salts with their three ions do not have thrice the osmotic pressure of equimolecular concentrations of cane sugar. This result led Arrhenius to postulate incomplete dissociation of electrolytes in solution.

Pfeffer's data on osmotic pressure were obtained for purely biological purposes, and it was not until 1887 that Van't Hoff brought forward his theory of solutions. Up to that period Van't Hoff had been engaged on the study of chemical equilibria in connection with chemical affinity, and from his observations gradually realized that the quantitative laws which apply to gases, and which fundamentally are very simple, also apply to dilute solutions.

Pfeffer's quantitative experiments had already shown that the osmotic pressure of solutions was directly proportional to their concentration, and taking a kinetic basis for osmotic pressure manifestations, the theoretical proof of Pfeffer's results quickly followed and took the same form as that used for gases. Further, from thermodynamical reasoning (the details of which can be obtained from the larger treatises on Physical Chemistry), Van't Hoff was able to prove that the osmotic pressure is directly proportional to the absolute temperature. Van't Hoff's theoretical conclusions were supported by Pfeffer's data. Later estimations, however, showed that in many cases Pfeffer's values were too low.

Since the osmotic pressure of a solution is directly proportional to its concentration, or inversely proportional to the volume of the solution, and since it is also proportional to the absolute temperature, it follows that if the osmotic pressure be

represented by P and the absolute temperature by T and the volume by V, then:

$$P \alpha \frac{T}{V} \quad \text{or} \quad PV = RT$$

This equation where R is a constant is similar to that employed to express the behaviour of a perfect gas. The value of R has been calculated and found to be almost exactly that which obtains for gases, i.e. $R = 83{,}900$ (in gram-cm. units). Thus Van't Hoff obtained two extremely important results, namely that the equation $PV = RT$ is valid for dilute solutions and that the numerical value of R is the same for dissolved substances as for gases, or in Van't Hoff's words: "the osmotic pressure exerted by any substance in solution is the same as it would exert if present as a gas in the same volume as that occupied by the solution, provided that the solution is so dilute that the volume occupied by the solute is negligible in comparison with that occupied by the solvent."

These various laws only apply if the solutions are sufficiently dilute, and do not hold with the same degree of accuracy in higher concentrations. In moderately high concentrations, the Van der Waal's correction:

$$\left(P + \frac{a}{V^2}\right)(V - b) = RT$$

may be applied. In this expression b represents the actual volume occupied by the molecules of gas, or, in the case of solutions, of the solute. With gases under low compression, or dilute solutions, the value of b is negligible and can be disregarded. But in the case of a gas under compression, although the total volume is diminished, the actual volume occupied by the molecules of the gas remains the same, and as the pressure upon the gas is increased, this volume must be taken into account. To effect this correction for the volume of the molecules of the gas, b must be subtracted from the total volume V occupied by the gas, and the expression $V - b$ is obtained.

It is known that the molecules of a gas exert an attraction upon one another, and the same fact applies to substances in solution. In very dilute solutions and gases under low pressure, there is little chance of one molecule coming into contact with a fellow molecule on account of the relatively large spaces that lie between

them. With gases under pressure and concentrated solutions, however, the spaces between the molecules become reduced and this attractive force a comes into play. The attractive force of molecule upon molecule will act in the opposite direction to b and has been found to be inversely proportional to the square of the volume occupied by a given number of molecules. Thus when a certain pressure is applied to a gas, two different forces will come in operation at one and the same time, for there is (1) the direct pressure effect to be taken into consideration and (2) the mutual attraction of the molecules for each other is increased, with the result that the final volume attained is slightly less than if the pressure were acting alone. P, then, in the simple gas equation, must be increased by $\frac{a}{V^2}$.

The Mechanism of Osmotic Pressure.—The exact mechanism of osmotic pressure is still in dispute. The old bombardment theory originally advanced by Van't Hoff must be discarded. Osmotic pressure on this theory was accounted for by the bombardment of the walls of the vessel by solute particles, in the same way as the pressure of a gas is produced according to the kinetic theory. Traube put forward the view that there was a sieve action of the semi-permeable membrane which enabled it to differentiate between one substance and another. Acting like a sieve, the semi-permeable membrane would prevent the passage of solute particles which have a relatively large volume. Surface tension, electrical action and mutual attraction of solvent and solute have all been invoked to explain the mechanism of osmotic pressure, but difficulties are encountered in every case. Another suggestion, and one that has been widely accepted, is that a solution and its solvent are not in equilibrium at the same temperature and pressure. When the temperature of the solvent is lowered until its vapour pressure is equal to that of the solution, the tension necessary to bring this equilibrium about is the osmotic pressure of the solution. A comparison of this kind between vapour pressure and osmotic pressure is based on the kinetic theory of molecular activity. On the surface of hot water, for example, the molecules are in such a state of activity that some escape into the air and form vapour. The greater the heat of the water, the greater the number of molecules of water that will escape as vapour from the surface, and in order to escape from the liquid, energy must be expended by the mole-

cules to overcome the surface tension of the liquid, water, in the particular case we are discussing. On the other hand, in the case of an aqueous sugar solution of similar surface area to the water, there cannot be so many water molecules at the surface, for sugar molecules will be occupying part of this space. If we consider the air between, say, a vessel containing pure water and one containing a sugar solution as the semi-permeable membrane, then water molecules will pass through it but not sugar molecules. As a result there will be a greater vapour pressure above the surface of the pure water than over the sugar solution. As the vapour pressure of the solvent (water) is greater than that of the solution (sugar plus water), water will pass where its vapour pressure is high, i.e. from the pure water, to where the vapour pressure is low, i.e. to the solution, In time the level of the pure water will fall and that of the solution rise. Osmotic pressure is an equilibrium pressure. It is as though a weight were laid on top of a solution separated from a solvent by a membrane so as to prevent more solvent entering the solution. If this weight be not sufficient then others must be added until equilibrium is reached. Osmotic pressure may be redefined as the pressure which restores equilibrium with the surrounding water.

The membrane of the living cell is not completely semi-permeable, for certain salts are able to enter and leave the cell (see Chapter IV). A completely semi-permeable membrane has yet to be discovered. Copper ferrocyanide acts as a semi-permeable membrane to cane sugar and a number of salts, but it is permeable to others, notably the halides of potassium.

METHODS OF DETERMINING OSMOTIC PRESSURE

Pfeffer's method of directly determining the osmotic pressure of a solution has already been discussed. There are several other methods available.

The *Plasmolytic Method* of de Vries can be used with success although the values obtained are as a rule too high. The initial decrease in volume of the cell is not due to plasmolysis of the protoplast but to the shrinking of the cell wall. In the turgid state the cell wall was held rigid by the osmotic pressure of the cell, and it is only when the wall has ceased to be in an expanded condition that the plasmolysing effect of the external solution can withdraw the protoplast from the cell wall.

Höfler* has introduced certain refinements into the original method. In Hofler's method, if P represent the osmotic pressure of the cell and P' that of the plasmolysing solution, and if the volume of the cell be measured before and after treatment with the plasmolysing solution,* the first contraction of plasmolysis can be discovered. In actual practice the cells are first immersed in paraffin and their volume measured. The cells are then transferred to the given solution in which limiting plasmolysis occurs and the volume once more determined. If V represents the volume of the cells before treatment and V' that after treatment, then:

$$P = \frac{P'V'}{V}$$

Other methods of determining osmotic pressure are the lowering of vapour pressure, the elevation of the boiling-point and the lowering of the freezing-point. It has been shown by thermodynamical reasoning that the lowering of the vapour pressure, elevation of the boiling-point and lowering of the freezing-point due to the addition of a definite quantity of solute to a definite volume of solvent are each proportional to the osmotic pressure of the solution. Moreover, equations have now been obtained for expressing the exact relationship between these three factors and the osmotic pressure. The precise method of experimental procedure can be obtained in practical text-books of organic and physical chemistry.

For obvious reasons the boiling-point method cannot be used for determining the osmotic pressure of cell solutions. The freezing-point method, however, has found extended application at the hands of Dixon and Atkins. The cell sap must first of all be expressed from the tissues, and the freezing-point is then discovered. The great objection to the method rests in the fact that the nature of the cell sap may not be the same in the expressed state as it is in the living cell. According to Dixon and Atkins,† to obtain a fair average sample of cell sap it is necessary, in the first place, to render the protoplast completely permeable by immersing the tissue to be investigated in liquid air, as at such low temperature enzymic and other cell reactions are negligible. Following upon this treatment, they have ascertained that the

* *Ber. deut. bot. Ges.*, 1917, **35**, 706.
† *Proc. Roy. Dublin. Soc., N.S.*, 1910, **12**, 275.

expressed sap of successive samples has practically the same osmotic pressure.

In cases where there is only a small amount of sap available, the neat and elegant method of Barger* can be used. With care this method gives reasonably accurate results. The method depends on the fact that if two solutions are placed in an enclosed space, and if the vapour pressure of one be greater than that of the other, vapour will pass from the solution with the higher vapour pressure into the one with lower vapour pressure until the two solutions reach equilibrium. For the actual determinations capillary tubes are drawn out and alternately filled with small droplets of expressed sap and cane sugar solution of known concentrations. The capillary tubes are then attached to a slide with Canada balsam and the drops measured under the microscope. If after a given time there has been no change in the size of the drops then they must have the same vapour pressure, and expressed sap and cane sugar solution are isotonic, and the osmotic pressure of the sap can be calculated.

ANOMALOUS OSMOSIS

In the various cases considered above in which a solution has been separated from the solvent by a membrane, the result has been that the solvent has passed through the membrane into the solution. The reverse of this phenomenon has now to be discussed, in which a solvent passes from a solution across a membrane into the solvent: this is termed *negative osmosis* or *anosmosis*.

Bartell and his co-workers† have carried out a number of investigations on negative osmosis. Membranes of gold-beaters' skin or porcelain were used. Bartell ascertained that when the pores of the membrane were $0 \cdot 9\mu$ in diameter no osmotic effects were shown. When the pore-size lay between $0 \cdot 4$ and $0 \cdot 1\mu$, and using a solution of magnesium chloride or sulphate, negative osmosis was exhibited. Water was withdrawn from the solution of the chloride or sulphate, the solution within the membrane became stronger, and there was a fall in osmotic pressure. When the pore-size was less than $0 \cdot 1\mu$, normal osmosis occurred. Water excreted from certain cells at night is due to this process of

* *J. Chem. Soc.*, 1904, **85**, 286.

† *J. Phys. Chem.*, 1911, **15**, 659; *J. Amer. Chem. Soc.*, 1914, **36**, 646; 1916, **37**, 1036; *J. Phys. Chem.*, 1920, **24**, 444, 593.

negative osmosis, the water coming from the solution in the cell vacuole.

According to Bartell, negative osmosis depends on the electrical charges on the membrane. In the case of solutions of phosphoric acid and water, it was found that the pores of the membranes were positively charged, and this gave rise to a negative charge to the entering water molecules. At the same time phosphoric acid dissociates in solution into PO_4''' ions and $3H\cdot$ ions. In the case of the acid radicle PO_4, three negative charges are concentrated on one ion. The influence of such a negatively charged ion will be greater than a hydrogen ion with its single positive charge. It has already been seen that the entering water molecules are negatively charged and will come into contact with negatively charged phosphate ions, and since a negative charge repels a negative charge, water will be forced out. A negative pressure as high as thirty atmospheres has been recorded in this way.

In the case of a solution of cerium chloride, $CeCl_3$, dissociation in water will lead to the formation of cerium ions, each carrying three positive charges, and negatively charged chlorine ions, each with a single charge. As in the last case, the entering molecules are again negatively charged, and here will be attracted by the positively charged cerium ions, and a positive pressure will be set up. Thus either a decrease or increase in pressure may be registered. It is important to bear in mind that these porcelain membranes are not semi-permeable in the sense used before, and that negative osmosis is only registered when the pores are of a certain diameter.

THE WATER RELATIONS OF THE CELL

If a cell be placed in water, water will enter the cell from outside and as a natural result the cell wall will suffer distension.* The wall of the cell, however, is elastic and will resist this distension. The distended wall compresses the protoplasm and cell sap, and the hydrostatic pressure of the protoplasm and cell sap (which is now called the *turgor pressure*) is equal and opposite at any moment to the inward component of the tensions in the cell wall. This turgor pressure will tend to force water out of the cell. When such a cell is fully distended and equilibrium has been attained,

* See Thoday, *New Phyt.*, 1918, **17**, 108, and Höfler, *Ber. deut. bot. Ges.*, 1920, **38**, 288.

the osmotic pressure of the cell sap will be balanced by the turgor pressure. The cell is now fully turgid and cannot absorb any more water. This state of affairs will last so long as either the osmotic pressure or the tensions of the cell wall suffer no alteration. Representing the turgor pressure of the cell by T and its osmotic pressure by P, we have under the conditions of equilibrium discussed above:

$$P = T$$

or

$$P - T = 0$$

The value $(P - T)$ has been termed by Stiles* the *suction pressure*

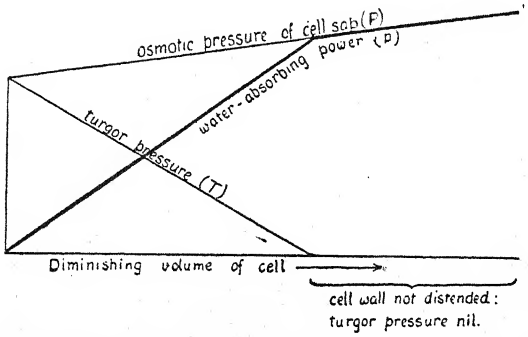


FIG. 6.—Curves showing the inter-relationships between osmotic pressure of cell sap (P), turgor pressure of cell wall (T) and suction pressure of cell (p). (After Thoday.)

of the cell. The suction pressure of a cell is the amount of osmotic pressure left over to draw water into the cell and is a measure of the water-absorbing power of the cell. The suction pressure of a cell is of fundamental importance in connection with the water-absorbing power of the root-hairs.

The connection between osmotic pressure, turgor pressure and the suction pressure of a cell is shown graphically in Fig. 6. As the turgor pressure falls, so the suction pressure rises.

When a cell is flaccid, the turgor pressure will be equal to zero, and in this case the osmotic pressure will be equal to the suction pressure. Thus the water-absorbing power of a completely flaccid cell will depend upon its osmotic pressure.

A further problem arises in this connection. Since the water-absorbing power of a cell is dependent upon its suction pressure,

* *Biochem. J.*, 1922, **16**, 727.

how will the situation between two adjacent cells in contact be affected? For example, if we have a cell A in contact with a cell B, and if P_A and P_B and T_A and T_B represent their osmotic and turgor pressures respectively, we shall have as the condition of equilibrium:

$$(P_A - T_A) = (P_B - T_B)$$

Whether cell A absorbs water from cell B or *vice versa* will depend on the values $P_A - T_A$ and $P_B - T_B$; if the former is greater than the latter then A will absorb water from B, quite irrespective of the absolute values of the osmotic pressures, P_A and P_B. Thus B may possess a greater osmotic pressure than A, yet because the turgor pressure is also greater it may be unable to remove water from A and in fact may yield water to it.

METHODS OF ESTIMATING SUCTION PRESSURE

Ursprung and Blum,* who have been responsible for a number of important investigations in connection with suction pressure, have described two distinct methods of determining its magnitude. In the first method they made use of the fact that since the suction pressure of a cell is the difference between the osmotic pressure and the turgor pressure, plasmolysis could be used for this purpose. The first step in the method is to measure the volume of the cell in liquid paraffin, and the second step to find a concentration of cane sugar solution which just does not change the volume of the cell, and lastly one that just does. The mean of the two values gives the suction pressure of the cell.

Some serious errors inherent in this method have been pointed out by Ernest.† The first error lies in the fact that the removal of a plant organ and its immersion in paraffin oil will immediately reduce the supply of water both to and from the tissues to zero. Further, the gradients of suction pressure that bring about movement of water from cell to cell are dynamic and not static gradients, and if for any reason the supply of water through the cells be cut off, the cells will begin to lose water to one another because a dynamic gradient will no longer be present and ultimately all the cells will have the same suction pressure. Another difficulty in this connection lies in the fact that, in the case of two adjacent cells with the same suction pressure, should one

* *Ber. deut. bot. Ges.*, 1916, **34**, 123, 525, 539; 1918, **36**, 577, 599; 1919, **37**, 453. *Biol. Zt.*, 1920, **40**, 193.

† *Ann bot.*, 1931, **45**, 717.

of these cells for any reason become disrupted its contents will be able to exert their full osmotic pressure, and this osmotic pressure will be greater than the suction pressure of the intact cell. The contents of the disrupted cell coming into contact with its uninjured fellow will draw water from the latter and as a consequence the suction pressure of the uninjured cell will rise.

The second method devised by Ursprung and Blum for determining suction pressure consists in determining the concentration of a solution which contains some substance to which the cell membrane is impermeable and brings about no change in cell volume. In this case the net value of the suction pressure will be zero, so that if P' represents the osmotic pressure of the external solution and P the osmotic pressure of the cell sap and T the turgor pressure, the following relations will hold:

$$P - P' - T = 0$$

or

$$P' = P - T \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

if the suction pressure of the cell when placed in distilled water be represented by S, then:

$$S = P - T \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

from equations (1) and (2) S and P' are both equal to $P - T$, and T has the same value in both equations, thus:

$$S = P'$$

The suction pressure of the cell is therefore equal to the osmotic pressure of some non-penetrating solution in which the cell remains unchanged in volume. In practice it is customary to use weight rather than volume for the determination of the suction pressure.

A simple method of estimating suction pressure has been introduced by Molz.* The plant tissue or organ of which the suction pressure is to be determined is severed from the plant and stored in paraffin oil in which it can remain for several hours without injury. For the actual estimation, cane sugar solutions of various concentrations are made up and placed in small bottles of 20 c.c. capacity. Appropriate strips are now cut from the tissue or organ under paraffin and the strips exactly trimmed so that their length can be determined under a micrometer scale. The length of the strips is first measured under paraffin, and

* *Amer. J. Bot.*, 1926, **13**, 433, 465.

the paraffin then quickly removed with a piece of filter paper, and the strips immersed in the various concentrations of cane sugar for ninety minutes. At the end of this period the length of the strips is once more measured, this time under cane sugar solution. The osmotic pressure of the solution which causes no increase or decrease in the length of the tissue gives the suction pressure of the cells.

VARIATIONS IN OSMOTIC PRESSURE IN PLANTS

A very large number of observations have been made on the osmotic pressure of plant tissues and a variety of different methods have been used.

Pfeffer considered that the osmotic pressure of land and fresh-water plants varies between 5 and 11 atmospheres. Dixon and Atkins,* after a large number of determinations using the freezing-point method, found this original estimation to be too low. According to these investigators, in only 13 cases out of 53 did the value fall below 11 atmospheres. Actually the highest osmotic pressure that has been recorded for the sap of a plant was 153·1 atmospheres for the marsh xerophyte *Atriplex confertifolia*.

The moulds Aspergillus and Penicillium have been "educated" to withstand solutions of high osmotic pressure by successive cultivation in progressively higher concentrations. The hyphae of these forms will burst if replaced in distilled water owing to the great development of suction pressure.

The osmotic pressure of plant tissues has been found to vary with position in the plant, and it has been found that there is a distinct tendency for the osmotic pressure to increase in the higher levels of a plant. In general terms it can be said that the osmotic pressure of woody plants is greater than that of herbaceous forms. Harris, Gortner and Valentine,† using the expressed sap of *Betula lutea*, *Quercus Prinus* and *Robinia Pseud-acacia*, obtained the following values:

	Height in Feet	*Osmotic Pressure in Atmospheres*
Betula lutea	66	15·55
	52	16·01
	39	15·12
	25	14·11
	11	12·63

* *Sci. Proc. Roy. Dublin Soc., N.S.*, 1913, **13**, 422.
† *Bull. Torrey Bot. Club*, 1917, **44**, 267.

	Height in Feet	Osmotic Pressure in Atmospheres
Quercus Prinus	47	20·23
	36	20·08
	30	19·72
	19	19·57
Robinia Pseud-acacia	51	12·44
	39	11·07
	29	10·87
	9	10·68

With the exception of *B. lutea*, there is a slight and progressive increase in the osmotic pressure of the leaves the higher their point of insertion on the plant. The slight exception recorded for *B. lutea* may well be due to an accident of determination.

Ursprung and Blum,* however, were unable to find this steady increase, so that this rule is by no means general. They investigated the osmotic pressure of leaves of *Fagus sylvatica* at different heights, the values of the osmotic pressure being obtained by comparison with a molar concentration of potassium nitrate. Although they ascertained that there was apparently no connection between height and increase in osmotic pressure, they did discover a rough gradient from the cortical cells to the interior in branch, stem and root:

Cortex—	
Inner	0·667
Outer	0·671
Phloem parenchyma	0·573
Companion cells	0·721
Cambium	0·64
Xylem parenchyma	1·008
Cortical medullary rays	0·808
Xylem medullary rays	0·954

In the case of hosts and their parasites (in this case angiospermic parasites), determinations have shown that the osmotic pressure of the parasite is higher than that of the host. With the parasite *Phoradendron californicum*, whose hosts can be either *Acacia Greggii* or *Olneya Tesota*, the following values have been recorded:

	Osmotic Pressure of Leaf Sap of Host in Atmospheres	Osmotic Pressure of Sap of Parasite in Atmospheres
Acacia Greggii	26·57	33·66
Olneya Tesota	25·24	26·98

* *Ber. deut. bot. Ges.*, 1916, **34**, 123.

According to Harris,* epiphytes have an abnormally low osmotic pressure in their cells. In the epiphytic forms examined by him the osmotic pressure was found to be from 37 to 60 per cent lower than the expressed sap of herbaceous plants.

E. Drabble and Lake,† and also E. Drabble and H. Drabble, as well as T. G. Hill,‡ have found that the osmotic pressure of root-hairs of halophytes is a good deal higher than those of mesophytes, whilst Iljin and his co-workers have estimated the osmotic pressure of the cells of roots and leaves of a number of different swamp, meadow and steppe plants, and discovered that in the roots the lowest osmotic pressure values occurred in swamp plants and the highest in steppe plants. It would therefore appear that the osmotic pressure of roots growing in soil of high water content (i.e. swamp flora) is less than that of a plant growing in soil with a lower water content (i.e. steppe plants).

Light has also a marked effect upon osmotic pressure of plant tissues. The stronger the light intensity the greater the osmotic pressure. The leaves of plants which live under shade conditions have a lower osmotic pressure than those of plants exposed to full sunlight. Algae in the presence of strong sunlight increase the osmotic pressure of their cells. For example, Bucheim§ in his observations on Cylindrocystis found that strong sunlight brought about an increase in the osmotic pressure of the cell sap. Dixon found a similar increase in osmotic pressure of leaves under the influence of strong sunlight, and attributes this, probably correctly, to increase in concentration of carbohydrates from photosynthesis. Copeland and de Vries have shown that the cell sap of etiolated plants is lower than normal ones.

There is also a periodicity in the value of the osmotic pressure of plant cells. Ursprung and Blum|| found that the values increased in the morning to the afternoon, and then fell away with the advance of night.

Substances in the Plant Responsible for Osmotic Pressure.—De Vries made an analysis of the individual substances present in the expressed sap of different plants, and calculated from the amounts present the osmotic pressure each would exert. Roughly speaking the osmotic pressure of the sap corresponded with the sum of the osmotic pressures calculated from his analytical data. De

* *Amer. J. Bot.*, 1918, **5**, 490.
† *New Phyt.*, 1905, **4**, 189; *Biochem. J.*, 1907, **2**, 117.
‡ *New Phyt.*, 1907, **7**, 133.
§ *Inang. Diss. Bern.*, 1915.
|| *Ber. deut. bot. Ges.*, 1916, **34**, 105.

Vries' results for the petiole of *Heracleum Sphondylium* are given below:

Organic acids (mainly malic and calculated as such) ..	0·013
Potassium salts of organic salts	0·013
Glucose (this term covering other sugars)	0·152
Sodium chloride	0·014
	0·192
Found saltpetre value of sap	0·220

It will be seen that the two values are approximately the same, but it must be admitted that the comparison is very rough.

THE MAGNITUDE OF SUCTION PRESSURE IN PLANTS

Ursprung and Blum* carried out an important series of investigations on the suction pressure of the roots and leaves of *Fagus sylvatica*, and found that considerable differences existed in the value of the suction pressure in different tissues of the same organ. The value of the suction pressure rose from the epidermis, guard-cells, spongy parenchyma to the palisade tissue for the leaf of *F. sylvatica*:

Distance in metres	*Epidermis*		*Guard Cells*	*Parenchyma*	
	Upper	*Lower*		*Spongy*	*Palisade*
2·7	3·9	7·5	9·3	11·0	15·0
8·7	8·4	9·3	9·9	12·4	15·6
13·0	9·9	10·5	10·5	14·3	17·1

Not only was an inward gradient of suction pressure found, but an upward gradient as well. In all questions of water transport from cell to cell, it is not the osmotic pressure but the suction pressure which is the important factor concerned, as it is the amount of the suction pressure that determines the direction of flow.

In the stem of *F. sylvatica*, the gradient of suction pressure was from within outwards, whilst in the root the reverse was the case, i.e. the lowest suction pressure was found in the piliferous layer. In *Vicia Faba*† and *Phaseolus vulgaris*, the root tissues again, as in

* *Ber. deut. bot. Ges.*, 1916, **34**, 539. † Ibid., 1921, **39**, 70.

F. sylvatica, showed a steady increase from the piliferous layer as far as the innermost layer of the cortex immediately abutting upon the endodermis, but the suction pressure of the endodermal cells was less than that of the cortical tissues, although higher than the pericycle within. According to Ursprung and Blum, this anomaly may possibly be due to the fact that the suction pressure as measured is an average suction pressure in cases where this value varies over the surface of the cell, and if the suction pressure on the outer side of the endodermal cells should be greater than the adjoining cortical cells, and lower in value than the pericycle cells on the inner side, the entrance of water to the stele can be explained.

Ursprung and Blum* also ascertained that in the leaves of Ilex which had been allowed to undergo a certain amount of desiccation, the suction pressure of all the cells showed a considerable increase in pressure owing to the loss of water. The suction pressure of the palisade cells was found to be particularly high, possibly owing to increase in osmotic pressure brought about by the formation of fresh osmotic substances in these cells.

In alpine species, Blum* found that on the whole the cells in the aerial region gave a higher suction pressure than the root tissues. The suction pressure of the floral organs was particularly high. Soil moisture was found to be the most important factor influencing suction pressure. These results have been confirmed by Molz,† who showed that both soil moisture and atmospheric humidity are important external factors influencing the suction pressure of cells. Heavy rain, for example, after a drought, may increase the suction pressure of root cells by as much as 20 atmospheres.

ABSORPTION OF WATER BY THE ROOT

It is only a special portion of the root in land plants that is responsible for the absorption of water and dissolved salts from the soil, namely the root-hairs. These root-hairs are delicate structures which grow out from the piliferous layer of the root. There is a more or less mucilaginous layer on their cell walls whereby they are able to adhere to the soil particles and make intimate contact. The older portions of the root are devoid of root-hairs, and they do not occur on the root cap and the region of cell division and enlargement. They extend as a general rule

* *Beihfte. Bot. Centralbl.*, 1926, **43**, 1. † *Amer. J. Bot.*, 1926, **13**, 433, 465.

from a distance of 1 to 4 cm., and are confined to a limited zone near the tip of the root, just above the region of active division. For further details of the structure and physiology of root-hairs, the review by Farr* should be consulted.

It is frequently stated that the function of root-hairs is to absorb water from the soil. Although this is correct, they possess another important function inasmuch as they increase the area of absorption of the root.

The root-hairs come into contact with the soil particles, whose surfaces are covered with thin films of moisture held by surface tension. In clay soils which are colloidal in nature, imbibition plays an important part in the retention of water by the clay. It

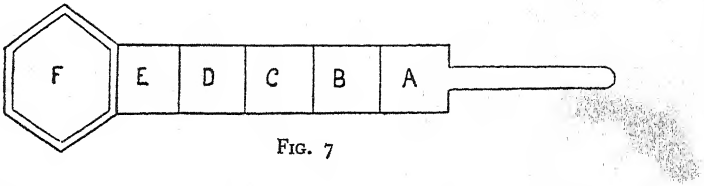


FIG. 7

has been ascertained that as the soil becomes more and more dry, these water films on the surface of the soil particles are held more and more firmly. These films of moisture have to pass from the surface of the soil particles into the root-hairs, and from thence into the cortical tissues and finally the stele. The suction pressure of the cells of these various tissues supplies the necessary pull, and brings about the entry of the water from the soil.

We have already considered the case of two cells in contact with one another and found that it is the suction pressure that allows of the entrance of water and not the osmotic pressure, and that when the cells are in equilibrium, their suction pressures will be equal. V. H. Blackman† has shown that in the case of a root-hair in contact with a number of cortical cells and finally a trachea (Fig. 7), if there be a gradient of suction pressure, water will enter the root-hair, pass from hence into the cortical cells and finally enter the xylem vessel. It is a gradient of suction pressure from root-hair to xylem that is needed. If the root-hair A has a high osmotic pressure and is exposed to water, and the cells B to E and the xylem vessel F have progressively lower pressures, water will pass from A to F from the soil. Thus A will take up water from the soil and become turgid, and its water-

* *Quart. Rev. Biol.*, 1928, **3**, 343.

† *New Phyt.*, 1921, **20**, 106.

absorbing power will fall below B; B will now begin to take up water from A, and in turn become fully turgid and its water-absorbing power will fall below C; and C will now begin to take up water from B, and so the process will continue until F is reached. As the cells of the root-hair and cortical tissues become more and more turgid and cease to have any water-absorbing power, F will be able to draw water from outside A, however low its osmotic pressure may be; and the force with which water will be drawn by F from the soil will depend entirely on the difference between the osmotic pressure of the solution external to A and the osmotic pressure of F.

THE SOIL AND SOIL MOISTURE

It is generally stated that it is only to a depth of 8 to 9 inches that the soil is suitable for plant growth. This is called the *soil* proper, and it gradually shades into the *subsoil* beneath, which in its turn passes by degrees into underlying rock. The true soil is formed by the process of weathering of rocks. Such agencies as frost, snow, rain and air break down the original rock into smaller particles, and thus gradually the rock is transformed into a suitable substratum for the support of plant life. After the primary break up of rock under the agencies described above, the solvent action of carbonic acid is of importance, and leads to the decomposition of felspars, which are double silicates of aluminium and sodium and potassium or calcium to aluminium silicate which forms the main basis of clay.

Soil can either be formed *in situ*, when it is termed a *sedentary* soil, or the eroded matter of rocks may be carried away by streams and rivers and be eventually deposited in other parts of the globe as fresh beds of soil. The lower plants, such as algae and lichens, also play an important part in the erosion of rocks. They are able to exist on the bare rock surface and to penetrate slowly, and eventually frost also effects an entrance and leads to the gradual break-up of the rock.

The first vegetation obtains its supply of mineral nutrients from the dissolved portions of the soil particles, but at death their decayed and decomposed tissues return their mineral substances to the soil. In this way succeeding vegetation has a double source of supply of inorganic matter. Should the dead vegetation lie on the surface of the soil, it will eventually suffer decay and

may form peat. If, however, it becomes mingled with the soil, and this is usually the case, it is decomposed and broken down under the agency of bacteria and fungi to give humus.

The chief source of soil moisture is rain. A part of this runs into the subsoil and may be carried away by drainage, and a portion is retained by the surface layers. The excess water which sinks into the subsoil is spoken of as *gravitational* water. Some of the water in the soil is retained as fine films round the soil particles or in the minute crevices that are present, and this is termed *capillary* water. The water retained by the soil colloids, e.g. clay, is called *hygroscopic* water. The problem of soil water is highly complex, more so than the simple classification given above would lead one to suppose. On the older view the water was considered to be held in the form of fine films round the soil particles, but the advent of later investigations, which have shown that the soil is a complex colloidal system, have materially altered the older point of view.

Keen* has demonstrated that the evaporation of water from sand, silt, china clay and ignited garden soil is a relatively simple phenomenon, and follows the known laws of diffusion and evaporation. Evaporation from the soil, however, is a more complex phenomenon, and there is some factor present which operates in making the relation between the soil and soil water of a different and closer nature than is the case with sand, silt, etc. Keen obtained his results by direct weighing. The weighed samples of soil were placed in vessels and suspended over sulphuric acid, and then weighed at intervals, and it was found that sand held its water least tenaciously and soil the most tenaciously. When the colloidal properties of the soil are destroyed, the nature of the curves for water loss become identical with those of sand and silt. Evidently it is the colloidal nature of the soil that is responsible for the complex water relations that have been discovered to exist. The matter has been further investigated by Keen, Crowther and Coutts,† who discovered that the evaporation of water from soil was controlled by two factors depending on (1) the soil-water relationships and (2) environmental conditions. Environmental conditions include such factors as the diffusion of water vapour of the soil and bulk air movements set up by the temperature gradient from the bottom to the top of the soil, the

* *J. Agric. Sci.*, 1914, **6**, 456; *J. Faraday Society*, 1922, **17**, 228.
† Ibid., 1926, **16**, 105.

cooling of the soil by evaporation and the lower density of the moist air.

L. J. Briggs and McLane* have attempted to find the relative force with which the different mechanical particles of the soil hold their water. The experimental technique used was simple. The soil was placed in metal cups and centrifuged at a rapid rate, a centrifugal force of 1,000 gm. being developed. The water was flung off the particles and a measure of their water-holding capacity obtained. The following values were recorded:

	Diameter of Particles in mm.	*Relative Water-holding Capacity*
Coarse sand ..	2·0 –0·25	1
Fine sand	0·25–0·05	1
Silt	0·05–0·005	12
Clay	0·005	57
Humus	—	57

It will be seen that the relative water-holding capacity increased from coarse sand to humus. Shull† has used the seeds of the composite Xanthium to ascertain the force with which soil moisture was attained. The seeds were weighed and placed in soils of different moisture content; they were allowed to remain until equilibrium had been reached, and were then weighed again. An increase in weight gave the amount of water taken up by the seeds. An attempt was made to measure the actual force exerted by the seeds to take up this water. The seeds of Xanthium possess testas which are semi-permeable, and the seeds were placed in different concentrations of sodium and lithium chloride solutions, the osmotic pressures of which could be calculated. The amount of water taken up in these solutions was then compared with the values obtained from the soil. Shull claimed that the osmotic pressure of the solution from which the same amount of water was withdrawn as from the soil gave a measure of the force with which that soil held its moisture. In one case it was ascertained that an initial force of nearly 1,000 atmospheres had to be exerted by the seeds to remove water from the soil.

There is a continuous battle between the water of the soil and the power of the root-hair to absorb this water. According to Ursprung and Blum,‡ the suction pressure of root cells quickly

* *U.S. Dept. Agric. Bur. Soils Bull.*, 1907, **45**.
† *Bot. Gaz.*, 1913, **56**, 169; 1916, **62**, 1; 1920, **69**, 361.
‡ *Ber. deut. bot. Ges.*, 1921, **39**, 70.

accommodates itself to the osmotic pressure of a solution to which they are transferred. Bean roots, for example, germinated in sawdust showed a suction pressure of 1·4 atmospheres, but when removed to a 0·65 per cent cane sugar solution of osmotic pressure 5·3 atmospheres, the value of the suction pressure rose to 5·7 atmospheres in just over three days. It is therefore possible that the suction pressure of root cells is a measure of the forces of the soil resisting the withdrawal of water, being just greater than these.

THE WILTING COEFFICIENT OF THE SOIL

It will have been understood from the previous discusson that the plant is not capable of using all the water present in the soil. The water that the plant can absorb from the soil is termed *growth water* or *available water*. The water that the plant is incapable of absorbing is sometimes spoken of as *unavailable water*, a term first used by Sachs, or it can be expressed as the *wilting coefficient** of the soil. The term "unavailable water" is not at all a happy one, for the wilting of the plant does not depend entirely on the amount of water present in the soil, but on a large number of other factors, such as the rate of transpiration and rate of absorption. The wilting of a plant does not necessarily signify that it is unable to withdraw water from the soil; it is simply an outward and visible sign that it is unable to withdraw water with sufficient rapidity for its physiological needs, for wilted plants are still able to withdraw water from the soil.

Sachs used tobacco plants in an attempt to estimate the amount of this so-called unavailable water. The plants were grown in different soils and the amount of water present at the beginning and end of the experiments was determined, the quantity left in the soil when the plants had wilted being the unavailable water. Some of his values are given below:

	Water at Commencement of Experiment Per cent	*Water at End of Experiment Per cent*
Sand and humus ..	46·0	12·3
Loam	52·1	8·0
Plain quartz sand ..	20·8	1·5

It will be seen that sand mixed with humus held more water than sand used alone.

* See Briggs and Shantz., *Bot. Gaz.*, 1912, **53**, 20.

Crump* working with moorland plants such as *Calluna vulgaris* and *Deschampsia flexuosa* has obtained more exact values:

Water Relations of Moorland Soils and Plants

Soil	*Calluna vulgaris*		*Deschampsia flexuosa*	
	Humus Per cent	*Non-available Water* Per cent	*Humus* Per cent	*Non-available Water* Per cent
Peat	54–78	27·0	74–80	54·0
Sandy peat ..	35	12·0	34·0	13·0
Loam	10–11	5·8	9·0	13·0
Sand	—	—	4·5	1·4

L. J. Briggs and Shantz attempted to determine the wilting coefficient of a number of different soils. This wilting coefficient is the percentage of water left in the soil after wilting has occurred. In all, twenty different soils and a hundred varieties of plants were investigated, the plants being of various types: xerophytes, mesophytes and halophytes. Most of these investigations, however, were concerned with the behaviour of wheat seedlings. The plants were grown in small pots, and the surface of the pots was then sealed with wax. Transpiration continued until eventually the plants wilted. Samples of the soil were then taken and the water content determined.

In one experiment (pot No. 74) the wilting coefficient of the soil was found to be 7 per cent, the death of the plant occurring twenty-eight days after the first wilting, when the amount of water was found to be 3·1 per cent. Thus the dead plant had still continued to absorb water and transpire it into the air. Up to this point the experiments of Briggs and Shantz had yielded valuable data on the subject of wilting and the absorption and transpiration of water by the plant when alive and also when dead. These two investigators now went on to make the remarkable claim that for every soil there is a definite wilting coefficient. For coarse sand they gave the value 0·9 per cent, for fine sand 3·6 per cent and for clay loam 16·6 per cent. Briggs and Shantz considered the wilting of the plant to be due to the mechanical nature of the soil. The moisture equivalent of the soil, which is

* *New Phyt.*, 1913, **12**, 125; *J. Ecol.*, 1913, **1**, 96.

the percentage of water which it can retain in opposition to a centrifugal force of one thousand times that of gravity, could be determined by the centrifuging method of Briggs and McLane (see above), and from this the wilting coefficient was calculated by the equation:

$$\frac{\text{Moisture equivalent}}{1 \cdot 84\,(1 \pm 0 \cdot 007)} = W$$

where W represents the wilting coefficient.

It has already been seen that wilting is brought about by the inability of the plant to absorb water from the soil with sufficient rapidity to keep pace with its transpiration rate, as well as other factors. Temporary wilting is frequently to be seen in soft-leaved plants, which will often flag on a hot summer's day and recover their turgor in the cool of the evening or at night; and this recovery takes place in spite of the fact that no water has been added to the soil. Briggs and Shantz make the process a static one. There is, however, no question of equilibrium to be considered at all in this matter; the whole problem centres round the power of the plant to absorb water from the soil to meet external expenditure by transpiration.

Caldwell* has seriously criticized the deductions of Briggs and Shantz in this respect. Working in the laboratories situated in the Arizona desert, Caldwell devised a series of pot-cultures of the same species in the same soil under three different environmental conditions: (*a*) desert conditions, (*b*) desert conditions, but plants kept in shade and (*c*) plants kept in a damp room. If the conclusions of Briggs and Shantz were correct all these plants should wilt when the same definite point of water limit had been reached by the soil in each case. Needless to say they did not. The wilting coefficient of the soil in which the plants had been grown under desert conditions was higher than the soil of the plants grown under shade conditions, and this again was higher than the soil of plants grown in a moist room.

Shive and Livingston† have compared the evaporating power of the air with the wilting coefficient of the soil. Plants were placed under different external conditions, and a special porous cup atmometer was used as a control. Graphically expressed, their results showed a well-marked curve, whereas if Briggs and Shantz were correct a straight line graph should have been

* *Physiol. Res.*, 1913, **1**, 1.

† *Plant World*, 1914, **17**, 81.

obtained. In this connection of the wilting coefficient of the soil, the review by V. H. Blackman* should be consulted.

The problem of wilting and transpiration is one of economic importance. Evaporation of water from different soils takes place at different rates on account of the variations in the forces with which different soils retain their moisture content. What is really required is a correlation between the evaporating power of the air with the moisture present in the soil. The investigations of Shive and Livingston are incomplete in this direction, and it would have been a matter of interest to have known the transpiration rate.

R. C. Knight† has ascertained the difference in water content of the leaves of different plants when turgid and also when in the wilted state, and shown that there is a constant difference of 1·5 per cent. If this amount of water be lost the leaves become flaccid, a result indicating the slight extensibility of the cell walls.

THE INFLUENCE OF EXTERNAL CONDITIONS ON THE ABSORPTION OF WATER BY THE ROOT

The quantitative data at present available are very unsatisfactory in this respect. There is no such thing as a unit of absorbing surface for the root. In practice it is quite impossible to measure unit-absorbing surface, as it is only the tips of the youngest roots and root-hairs that are capable of absorbing water from the soil.

Temperature.—This factor markedly affects water absorption from the soil. As the temperature of the soil rises, the plant is able to absorb water more rapidly. Moreover, the viscosity of the water is reduced and it can move at an increased rate in the soil.

Adequate aeration of the soil is necessary for the normal growth of plants. Certain types of plants, however, can grow in situations in which the supply of oxygen to the roots is limited. Aquatics in still water and bog and marsh plants can grow under poor conditions of aeration. As a general rule, the chief anatomical feature of such plants is an intense development of air spaces in the cortical region of the root.

As far back as 1907, F. E. Clements recognized that the air content of the soil atmosphere was an important factor in all soils, especially acid soils, and the view that sufficient aeration is a

* *J. Ecol.*, 1914, **2**, 43.

† *Ann. Bot.*, 1922, **36**, 361.

prime necessity for successful plant growth has gradually come to the fore ever since. Balls, for example, showed that the roots of the cotton plant were locally asphyxiated in water-logged soils, and death and decomposition followed in a few weeks. Similarly Harrison and Aiyer* arrived at the same conclusion for the rice crop in India. A. Howard and Singh,† as well as A. Howard and G. Howard, have shown that indigo wilt was due to insufficient aeration of the soil. If floods bring about a rise in the ground water, or if heavy rainfall waterlogs the surface soil for prolonged periods, the defective aeration which results from these conditions makes root regeneration difficult and wilt ensues. In confirmation of this view, it has been shown that other deep-rooted species exhibit wilt under these conditions, whereas shallow-rooted species do not. Moreover, wilt is common in years of heavy rainfall, and rare and of slight importance in dry years, and lastly it is seldom found on porous soils.

BIOLOGICAL ADVANTAGES OF OSMOTIC PRESSURE

If the presence of a cell vacuole containing dissolved substances be granted in plant cells, then these cells must show osmotic pressure. The only manner in which a plant could prevent its cells having any osmotic pressure would be to form colloids. It will be recalled that the intake of water in meristematic tissues is primarily due to imbibition and not osmotic pressure. The mechanical rigidity of the softer portions of the higher plants is maintained by the osmotic pressure of the cells. As long as the turgor of the cells is normal, the tissues remain firm, but when the supply of water is lowered in any way and turgor reduced, the tissues become flabby. Growth is also influenced by osmotic pressure, for it is only turgid cells which are capable of active growth.

* *Pusa Mem. Chem. Sci.*, 1913, **3**, 65.
† *Agric. J. India*, 1918, **13**, 36; 1919, **14**, 377; *Mem. Dept. Agric. India*, 1920, **11**, 1.

CHAPTER IV

PERMEABILITY

THE passage of substances into the living cell forms a large and fundamental problem of physiology, and comes under the general heading of the permeability of the cell. It is a well-known fact that protoplasm is permeable to many substances, such as gases, electrolytes and non-electrolytes. Unless this were the case, metabolism as we know it could not take place, and the cell would sooner or later die.

As long ago as 1884, de Vries, and a few years later (1888) Klebs, showed that plant cells are permeable to glycerol. When the cells were first placed in a solution of glycerol, plasmolysis occurred; but after some hours, recovery took place. This recovery of plasmolysed cells is termed *deplasmolysis*. De Vries and Klebs both correctly interpreted their results by considering that on deplasmolysis the glycerol had entered the cell and equilibrium had been attained. Such substances as cane sugar, amino-acids and inorganic salts only enter the healthy protoplast slowly. On the other hand, urea, acetamide, succinamide, sulphonal, caffeine and antipyrin enter fairly rapidly, whilst the protoplast is extremely permeable to ethyl alcohol. The penetration of a 0·5 molar solution of ethyl alcohol is so rapid that no plasmolysis takes place.

The major problem of cell permeability is bound up with the nature of the membrane which surrounds the protoplast. The existence of such a membrane has been denied. The objection that led to the denial of the existence of such a bounding membrane, largely arose from the fact that, as the living cell is dependent on a supply of substances for active metabolism from the external solution surrounding it, the presence of a semi-permeable membrane would prevent substances from being washed out of the cell, and must also prevent them effecting an entrance. It has been suggested that imbibition by cell colloids would account for the phenomena, rather than the presence of a definite membrane. But against such a view there remains the fact that the living cell behaves towards, say, a solution of cane sugar in exactly the same way as an artificial cell with a membrane of copper ferrocyanide containing a solution of cane sugar. If

such an artificial cell be placed in an isotonic solution of cane sugar, water will neither enter nor leave the "cell," because the two solutions are in equilibrium. On the other hand, if we immerse our cell in a hypertonic solution of cane sugar, water will pass out to the surrounding solution until equilibrium is once more restored; or again, if it be immersed in a hypotonic solution of cane sugar, water from the surrounding solution will enter the cell until equilibrium is reached. It is not necessary that the two solutions on either side of the membrane be the same to bring about the results described above. Imbibition follows different rules. Equal concentrations of electrolytes have a different effect on imbibition, depending on their action on the properties of water; and the proportional distribution of water between the two phases of the colloidal system will depend upon the nature of the electrolytes. The various conflicting results that have been obtained on this point can most probably be explained by the supposition that the membrane surrounding the protoplast is sometimes permeable to crystalloids and sometimes not. These remarks about the membrane surrounding the protoplast do not apply to non-vacuolated cells. The evidence here is very conflicting.

The exact nature of the membrane has given rise to considerable controversy, and the variety of terminology used by different workers has led to much confusion. The surrounding membrane will be referred to here as the *plasma-membrane*.

Overton, after a large and varied number of experiments, arrived at the conclusion that the plasma-membrane was composed of some fat-like material, hence his idea of the plasma-membrane came to be called the *Lipoid Theory of the Plasma-membrane*. He considered the plasma-membrane of fatty materials or substances closely allied to fats, such as lecithin and also cholesterol,* to form a thin film surrounding the cytoplasm. According to Overton, substances which are soluble in fats pass through the plasma-membrane into the protoplast, whilst those that are insoluble are unable to do so. This theory at one time received a good deal of support, but has now been abandoned. It is a well-known fact that inorganic salts enter the living cell, and these are compounds which are insoluble in fats. At the time Overton published his results, the only aniline dyes known

* Cholesterol is not a fat, or related chemically to the fats, but occurs in the so-called "unsaponifiable residue" of fats (see Chapter IX).

were soluble in lipoid substances. This is, however, no longer the case; a number of dyes are now known which are freely soluble in lipoid substances, and yet will not enter the cell, and conversely the greater number of dyes are insoluble in lipoid substances, and do enter the cell.

Overton worked by plasmolysis; if plasmolysis occurred, he concluded that the substance had failed to enter the cell. This is not necessarily the case. Plasmolysis is not a criterion of the non-entry of a substance into the protoplast. All that plasmolysis denotes is that the substance has not entered fast enough to cause equilibrium on the two sides of the plasma-membrane. The time factor must be taken into consideration in this connection, for it will take a certain time for deplasmolysis to occur.

De Vries, in his plasmolysis experiments using potassium nitrate, fully recognized this fact. He found that plasmolysis disappeared after a given time, in every case where the strength of the solution was not too great. Tröndle also showed that the leaf cells of Tilia when left overnight in solutions of sodium chloride of 0·2–0·5 molar strength, at first plasmolysized, but on inspection next morning all the cells showed recovery. It is clear that the salt must have entered the protoplast and increased the osmotic pressure of the cell sap, with the result that recovery had occurred.

Although hypertonic solutions of cane sugar are able to cause plasmolysis, yet leaves fed with solutions of cane sugar form starch. The cane sugar enters the cells very slowly. Tröndle,* for example, has shown that it takes thirty-six hours for a solution of cane sugar to enter the cells of Tilia leaves, whilst sodium chloride can bring about deplasmolysis in twenty-four hours. In experiments concerned with plasmolysis and permeability, it is better to employ the salt of divalent metals, such as calcium chloride, rather than the salt of a monovalent metal, as entry into the cell in the case of the former is slower and can be followed more easily.

There is a further point in this connection that must be kept in mind. Osterhout† has shown that it is easy to observe deplasmolysis in cells of Spirogyra with solutions containing such ions as $K^{\cdot}$, $Na^{\cdot}$, $Li^{\cdot}$, NH_4, $Ca^{\cdot\cdot}$, $Mg^{\cdot\cdot}$ and $Al^{\cdot\cdot\cdot}$, but the observations must be made continuously. There is preliminary plasmolysis,

* *Ber. deut. bot. Ges.*, 1909, **27**, 71; *Jahrb. f. wiss. Bot.*, 1910, **48**, 171.
† *Science, N.S.*, 1911, **34**, 187.

followed in course of time by deplasmolysis, and deplasmolysis is in turn followed by "false plasmolysis" in which the protoplasm appears as a shrunken mass away from the cell wall. This second plasmolysis is due to injury of the cells and has nothing to do with true osmotic plasmolysis. Thus, if observations were made at the first stage of true plasmolysis, and not again until false plasmolysis had occurred, the inference would be drawn that plasmolysis was permanent, and no penetration had taken place. It is possible that Overton was led astray under his experimental conditions on this account.

Nathansohn has brought forward a suggestion that the plasma-membrane is composed of a mosaic of lipoid and protein material, responsible respectively for the intake of lipoid-soluble and protein-soluble substances, for the entry of these two types of compounds is not independent of one another. This theory is generally spoken of as the *Mosaic Theory of the Plasma-membrane.* There are solution theories to explain the differential behaviour of membranes. The so-called "sieve" theory of Traube has been considered already in Chapter III.

There is yet another theory that must be mentioned, namely, the chemical theory. By the chemical theory, the membrane is considered to combine chemically with the substance to which it is permeable. The reaction is supposed to be reversible, so that on the interior of the membrane, the compound formed between the membrane and diffusing substance breaks down, with the result that the diffusing substance is set free on the other side of the membrane.

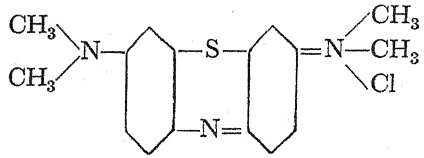
DETERMINATION OF THE PERMEABILITY OF PLANT CELLS TO SOLUTES

A number of methods have been used to measure the permeability of cells, but it must be confessed that most of them are of very doubtful merit.

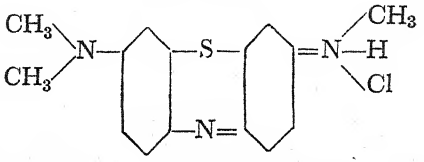
(1) *Dyes.*—It has been shown that certain dyes are able to enter the living cell. If filaments of Spirogyra be suspended in dilute solutions of methylene blue, the entrance of the dye into the cells can be observed after a time. The objection to this method lies in the fact that it not only shows entry but also accumulation. The dye is used in such high dilution that it is only after accumulation has occurred that its presence in the

cell can be seen, so that the concentration inside the cell must be very much higher than that of the external solution.

It has been recognized for some time that basic dyes enter the living cell very much more readily as the free base than in the form of their salts. A curious fact has been noted in this connection with regard to methylene blue, which is a basic dye and yet enters the cell at a low pH,* i.e. in acid medium, when it must be in the form of a salt. According to Irwin, ordinary methylene blue is a mixture, and always contains small amounts of its lower homologue, azine blue, which is less basic in nature. It is there-



Methylene blue



Azine blue

fore capable of existing as the free base in an acid medium to a certain extent, and it is azine blue which enters the cell in media of low pH and not methylene blue. On injury of the cell, however, methylene blue can enter at a range of pH from 5·5 to 9·5. This work of Irwin's explains the otherwise contradictory results of M. M. Brooks, who stated that methylene blue penetrates the cells of *Valonia macrophysa* with rapidity at a range of pH of 5·0 to 9·0, and who concluded that methylene blue was one of the few basic dyes which could enter the living cell as easily in the form of its salts as the free base. Brooks, however, still maintains that the permeability of Valonia to methylene blue depends on (*a*) the pH of the surrounding medium and (*b*) temperature, and that the azine blue found by Irwin is formed by oxidation from methylene blue, as it may be found in sap that has been expressed and allowed to stand for some time. It is difficult to say which of these views is correct, but it would appear that Irwin's results are the more reliable.

* For a definition of pH see Appendix.

(2) *Change in Colour of Cell Pigments.*—The anthocyanin pigments which occur in the dissolved condition in the cell sap of some plants show a colour change in acid or alkaline medium, and on this account have been used as indicators for the entrance of one or other of these groups into the cell. De Vries, for example, showed by the change in colour of the pigment in the cells from red to blue that ammonia penetrates the root cells of red beet.

(3) *Metabolic Method.*—In this method the cell is fed with different substances, such as cane sugar, glycerol, etc., and the formation of starch observed. An obvious disadvantage lies in the fact that if the external solution be too strong, water will be drawn out of the cell and the sugar concentration within may be increased, with the result that starch formation will occur independently of entry of sugar from the external solution. According to Iljin, the salts which penetrate a cell have the power of promoting the hydrolysis of starch and other polysaccharides in general. The result is the formation of soluble organic substances within the cell which raise the osmotic pressure to a value higher than would be attained simply as the effect of the entrance of the salt itself. Salt solutions in which failure of recovery from plasmolysis takes place fail to promote the hydrolysis of starch. The salt content of the cell must therefore be taken into account when such a method as this is employed, for the subsidiary hydrolytic action of the salt would play an important part in the amount of starch formed.

(4) *Microchemical Tests.*—The entry of substances into a cell may be detected by testing with suitable microchemical reagents. Janse fed cells with potassium nitrate, and was able later to detect the presence of nitrate with diphenylamine and potassium with platinic chloride.

(5) *Analysis of Cell Sap.*—Certain plants, such as the alga Valonia, possess cells of such a large size that the sap of a single cell can be expressed and analysed. In Valonia, it has been found that the cell sap contained a higher concentration of potassium than the surrounding sea water, but only traces of magnesium could be found in the sap, although the concentration of magnesium in sea water is considerably higher than potassium. The amount of nitrate in the vacuole was also large, although but a trace occurs in sea water. The alga Nitella has also been used in this way for permeability experiments. If the internodal cells be broken, sufficient sap may be obtained for analysis.

(6) *Analysis of the Plant.*—Seedlings are grown in culture solution, and an analysis is subsequently carried out on the whole seedling. Definite information of entry can be obtained in this way.

(7) *Analysis of External Medium.*—Culture media can be analysed after the plants have been grown in them for a given time. If various substances are found to be absent or in lower concentration than in the original medium, it is clear that they must have entered the plant. In such experiments as these, every care should be taken to keep the solution sterile and free from bacteria and fungi.

(8) *Changes in Electrical Conductivity of External Solution.*—Measurement of the electrical conductivity of the external medium can be used as a measure of the permeability of the cell membranes to the electrolytes within the cell. Plant tissues placed in distilled water or in a solution of a non-electrolyte will yield electrolytes to the surrounding medium by exosmosis and increase the electrical conductivity of the latter. If the external solution should contain a non-electrolyte, its presence will depress the conductivity and allowance must be made for this factor in comparative experiments. The method can also be used to measure the intake of electrolytes by the plant tissue; but the situation here is complicated by the factor of exosmosis from the cells, and intake and excretion of electrolytes will affect the conductivity in opposite directions. If allowance be made for exudation of electrolytes by exosmosis, quantitative results can be obtained. The method was successfully used by Stiles and Kidd in their investigations in the absorption of salts by carrot root.

(9) *Change in Weight or Volume of Turgid Tissue.*—Fully turgid tissues are immersed in a solution of substance sufficiently strong to produce a diminution in volume of the cells, but not sufficiently concentrated to bring about plasmolysis. The entrance of the dissolved substance into the cells will bring about a gradual increase in volume and also weight. Lundegårdh* employed this method to determine the entrance of salts into the roots of *Vicia Faba*. The roots were first immersed in a salt solution of such a concentration that contraction in length was brought about. As the salt entered the cells of the root, this initial contraction was followed by an increase in length. The reciprocal

* *K. Svenska Vetenskaps Akad. Handlingar*, 1911, **47**, No. 3, 1.

of the time taken by the root to increase in length from 25 per cent to 75 per cent of the total increase it underwent, was taken as an approximate measure of the rate of entry of the salt.

(10) *Methods depending on Plasmolysis.*

(*a*) *Tröndle's Method.**—In this method, pieces of tissue which were assumed to be similar, were immersed in solutions of the substance of various concentrations. The portions were examined at intervals to discover when plasmolysis had just occurred. On the assumption that the concentration of the solution was xN, and that plasmolysis occurred after a lapse of ten minutes, and that after a further interval of ten minutes the concentration was yN, the assumption was made that an amount of substance had entered the cells sufficient to raise the concentration of the substance in the cell sap by the value $(y - x)N$. The method, of course, ignores exosmosis. Tröndle considered that exosmosis has no effect on the entry of substance into the cell, which is a very doubtful assumption. The method also ignores the influence of the external concentration of the substance on its rate of entry into the cells.

(*b*) *Höfler's Method.*†—This is also a plasmolytic method, but the volume of the cells is measured as well. A definitely hypertonic solution is employed, of which the concentration is C. This will bring about a degree of plasmolysis p' (measured by the decrease in the volume of the cell). After a given time t, there will be a new degree of plasmolysis p''. Then if the osmotic concentrations of the cell corresponding to the two degrees of plasmolysis be respectively C' and C'', we have:

$$C' = Cp'$$

and

$$C'' = Cp''$$

from which we obtain

$$C'' - C' = C(p'' - p')$$

This equation gives a measure of the rate of entry of the substance into the cell.

(*c*) *Fitting's Method.*‡—Fitting, working with *Tradescantia discolor*, also employed the plasmolytic method in his permeability experiments. Various concentrations of a given solution were taken and pieces of tissue immersed therein, the tissue all being taken from the same part of the plant. The assumption

* *Biochem. Zeit.*, 1920, **112**, 259.
† *Ber. deut. bot. Ges.*, 1917, **35**, 706; 1918, **36**, 423; 1919, **37**, 314; 1920, **38**, 288.
‡ *Jahrb. f. wiss. Bot.*, 1915, **56**, 1.

was made here that the osmotic pressure would be of the same value throughout the tissue. The degree of plasmolysis was measured and the plasmolysis of 50 per cent of the cells taken as a standard. The plasmolysis in the next higher concentration was then ascertained, the same standard as before being employed, i.e. plasmolysis of 50 per cent of the cells. It follows that when 50 per cent of the cells of the second solution have suffered plasmolysis, some of the cells in the first solution will have recovered, i.e. deplasmolysed. The difference in the degree of plasmolysis is taken as giving an idea of the permeability of the cells. The method is not a satisfactory one. Exosmosis is not taken into account, and Fitting considers that this may be neglected if the tissue be given a preliminary immersion in distilled water, an assumption that is probably not valid.

(11) *Tissue Tension Method.*—The tissue tension method was first described by S. C. Brooks.* Strips of dandelion (*Taraxacum officinale*) scape are attached by one end to pieces of split cork, and the strips are horizontally placed so that they are free to move in a horizontal plane. The movement of the strips is then observed under a micrometer scale. For the actual experimental procedure the strips were immersed in 20 c.c. of the solution to be tested (in this case potassium nitrate) in which there was no appreciable curvature. The concentration was then increased by a known volume of a molar solution of the salt. The strip now underwent a decrease in curvature, which, however, soon ceased and was followed by an increase. The time that elapsed between an increase in concentration and the instant when the strip regained its original curvature was termed the *time of recovery*. Immediately upon recovery the concentration of the solution was once more increased and the time of recovery again noted. This process was repeated several times and in this way a series of times of recovery were obtained. An empirical value for the rate of penetration was ascertained by dividing the change in concentration by the time of recovery. Curves were obtained by plotting the rates of penetration and the times that had elapsed between the first immersion of the tissue in the solution and the middle of each recovery time.

None of the methods described above, with perhaps the exception of the last, measure the permeability of the cell as such, and most of them only give a qualitative measure of the entry

* *Amer. J. Bot.*, 1916, 3, 562.

of substances into the cell. Moreover, there are a number of disturbing factors that must be considered in connection with these various plasmolytic methods of determining permeability such as osmotic changes in the cell during the experimental period, brought about by causes other than penetration, exosmosis of osmotically active substances, injury to the protoplast from plasmolysis by too long contact with hypertonic solutions, etc. If the concentration of a gas or other substance on the two sides of a membrane be C' and C'', then the time necessary for the gas or other substance to diffuse through the membrane so as to give rise to the above concentration, i.e. so that equilibrium may be established, will give as the unit of permeability the amount thereof in unit time across unit area when a unit difference of concentration exists across the membrane.

It is not a difficult matter to measure the concentration outside the cell, the difficulty is to discover the concentration within. A number of investigators have neglected the difference of concentration on the two sides of the plasma-membrane, since it is this difference in concentration which is the cause of the drive of substances to the interior. The unequal distribution of salts inside and outside a cell were at one time explained either by combination with, or adsorption by, some of the cell constituents. There is, however, another possibility that must be taken into account, namely, the inability of some of the ions or ion to pass the cell membrane. According to Donnan, in such circumstances an unequal distribution of all the ions present is not due to any cause connected with the impermeability of the cells to the entry of the ions of the salt or to the salt itself, but to the presence of a non-diffusible ion. This is called the *Donnan Equilibrium.*

Consider the case of two solutions containing electrolytes, separated by a membrane through which one of the ions cannot pass. For example, large-sized molecules do not readily pass through animal membranes. If they give rise to a diffusible and a non-diffusible ion, the non-diffusible ion will hold on the same side of the membrane an ion of equal but opposite electrical charge. When a state of equilibrium has been reached on either side of the membrane, there will be an inequality in the distribution of the diffusible electrolytes. But the product of the concentration of any pair of diffusible cations and anions on one side of the membrane must be equal to the product of the concentrations of the same pair on the other side. Equilibrium

cannot be attained by an equal distribution of diffusible ions on the two sides of the membrane, because in such circumstances free electrical charges would be left on the side with the non-diffusible ion, and the sum of charges on the two sides must be the same. If P^+ be such a non-diffusible ion then we can represent a simple Donnan equilibrium by the following scheme:

yNa^+ yCl^-	Membrane	P^+ Cl^-
		xNa^+ xCl^-
(*a*)		(*b*)

In this system we shall have the relationship:

$$(Na^+)_a \times (Cl^-)_a = (Na^+)_b \times (Cl^-)_b$$

In (*a*) the concentration of sodium and chloride ions will be equal, but in (*b*) the chloride ions will be in excess of sodium ions. It has been shown by Donnan that as a result of these different concentrations of ions on the two sides of the membrane there will be a potential difference on the two sides. In living organisms such membranes are present with inequality of non-diffusible ions on the two sides and giving rise to an inequality in the distribution of electrolytes.

(12) *The Determination of Permeability to Dissolved Substances by the Direct Measurement of the Electrical Conductivity of Living Tissues.*—This is a method which has been elaborated by Osterhout,* who regarded the electrical conductivity of tissues as a measure of the permeability of the protoplasm. Osterhout considered the electrical resistance of plant or animal tissues to be an indicator of what he terms the normal "condition of vitality." Toxic agents cause changes to take place in this resistance. A large part of Osterhout's work on this particular method has been concerned with the stipe of *Laminaria Agardhii.*

In practice, the stipe of *Laminaria Agardhii* is cut into a series of discs or coins with a cork borer and packed together like a roll of coins. The column of discs is kept in position by means of two glass rods. At each end of the column of discs, and separated from it by a small length of solution, is a platinum electrode coated with platinum black and sealed into a glass tube filled with

* *Science, N.S.*, 1912, **35**, 112; 1913, **37**, 111; *Biochem. Zeit.*, 1914, **67**, 273; *J. Biol. Chem.*, 1918, **36**, 557; *J. Gen. Physiol.*, 1919, **1**, 515; 1921, **4**, 1.

mercury into which dips a copper wire connected with a Wheatstone bridge. The glass tubes into which the platinum electrodes are sealed are contained in special electrode holders made of hard rubber. About one hundred discs are used for each experiment. Repeated determinations on the same discs immersed in sea water showed by their constancy that even prolonged treatment did not damage the cells, for injury at once became apparent by a fall in the resistance. In one case the resistance of such a cylinder of discs was found to be 1,100 ohms, whereas that of a cylinder of sea water of equal dimensions was found to be 320 ohms. From this Osterhout concluded that the additional resistance must have been due to the living protoplasm and the cell walls, for the salts within the cells are in every case the same as sea water. Osterhout considered that further evidence in support of his views that the difference in resistance was due to the interposition of living cells, came from the fact that when the discs were killed with a 2 per cent solution of formalin in sea water or by careful drying the resistance fell to 320 ohms. This observation was also thought to show that ions penetrate into dead more readily than living tissue.

The question naturally arises, How far can electrical resistance be considered as a measure of permeability? Stiles and Jørgensen,* in a discussion of this point, have raised a number of objections to the unqualified acceptance of electrical conductivity as an exact measure of permeability. The electrical conductivity of the cells of a piece of tissue will naturally depend on a number of phases existing in the individual cells, and the various possible changes in these different phases must all be taken into account when the conductivity alters. The fact that dead tissues behave in a different way from living material affords no good evidence that the changes observed in the living tissues are not in any way connected with the cell wall. In the present state of our knowledge it is quite impossible to say dogmatically that the cell wall remains unchanged after death.

UNEQUAL ABSORPTION OF IONS BY PLANT TISSUE

The root system of the higher plants is exposed to a very dilute mineral solution, the soil solution, which is also highly ionized. To a limited extent roots show a selective power of absorption,

* *Bot. Gaz.*, 1918, 65, 526.

but at the same time they also take in a number of substances that do not appear to play any prominent part in metabolism. The question arises here as to whether these various ions enter the root cells independently of their partners. This has been found to be the case. A solution of potassium chloride contains potassium ions and chlorine ions, but the potassium and chlorine ions do not necessarily enter the plant cell together. In water cultures, for instance, a change in reaction is shown, the solution becoming either acid or alkaline, and this condition is brought about by the difference in the rate of entry of the various ions composing the culture solution. The entrance of one ion into the living cell cannot, on account of the attraction of the oppositely charged ions, take place without the excess being replaced by an equal quantity of another ion carrying an identical charge. Further, water itself is ionized to a small extent into hydrogen and hydroxyl ions:

$$H_2O \rightleftharpoons H^{\cdot} + OH'$$

Either hydrogen or hydroxyl ions, depending on the nature of the charge on the entering ion derived from the solvent, accompany the excess of absorbed ion into the tissue. Thus, if the entering ion carries a positive charge it will be accompanied into the cell by hydroxyl ion, and if it carries a negative charge, hydrogen ion will enter along with it. There is another possibility that must be considered in this connection, namely, that an equivalent quantity of some other ion carrying the same charge as the entering ion may diffuse out of the tissue.

As long ago as 1871, Wolff showed for the aquatic Lemna that the salts present in the plant and those in the surrounding medium were not the same, nor were they in the same concentrations. Substances which are present in very minute amount in the external medium are frequently present in considerable concentration in the plant. Iodine, which occurs in sea water to the extent of one part in a million, is found in the thalli of the Phaeophyceae in very much higher concentration, and at one time these plants formed the sole source of the iodine of commerce. In general terms, the concentration of salts in an angiospermic parasite are greater than in the host plant:

		Per cent
Poplar	K_2O	6·6
Mistletoe on *Poplar*	K_2O	16·6

		Per cent
Robinia	K_2O	2·3
Mistletoe on *Robinia*	K_2O	15·9
Fir	K_2O	8·4
Mistletoe on *Fir*	K_2O	30·7

Pantanelli and Sella* working with *Cucurbita Pepo* have shown that the roots exhibit an unequal rate of absorption of ions. These results have been extended to a number of other plants, showing that the unequal absorption of ions is an almost universal phenomenon.

Redfern† has investigated the problem of the unequal absorption of ions by the roots of pea seedlings (*Pisum sativum*) and of *Zea Mays*. The plants were grown in water culture. Some of her figures for the absorption of the ions of calcium chloride by *P. sativum* are given below:

	Calcium *Per cent taken up*	*Chlorine* *Per cent taken up*
After 36 hours	17·7	3·6
After 40 hours	12·8	4·0
After 60 hours	11·6	3·9

It was found that the divergence between the absorption of the two ions became less with the greater dilution of the solution. It will be observed that the calcium ion appears to enter the roots more rapidly than the chlorine ion. Theoretically the solution should have shown an acid reaction, owing to the combination between hydrogen ion of the solvent and chlorine ion left in excess in the external solution. Actually the solutions used in this work were found to remain nearly neutral, owing to the equivalent diffusion out from the cells of magnesium and potassium ions. Thus the excess of calcium ion absorbed by the roots in this experiment was replaced by the diffusion out of the tissue of ions carrying the same charge, and not by hydrogen ions from the water of the culture solution.

Similarly, A. R. C. Haas and Reed‡ found for Citrus seedlings that the two ions of a salt were not absorbed in equivalent amounts. In nearly every case more cation was absorbed than anion, a result confirming the work described above. The amount of ion absorbed depends on the nature of the other ion of the salt. Thus more cation was found to be absorbed from the nitrate than

* *Rend. R. Accad. Lincei, Cl. Sci., fis. mat. e nat.*, 1909, **18**, 481.
† *Ann. Bot.*, 1922, **36**, 167.
‡ *J. Agron.*, 1925, **17**, 577.

from the chloride of the same metal. The absorption of calcium was discovered to be retarded by the presence of potassium, and sodium produced a similar but less intense effect. In all cases an exchange of ions between roots and external solution was observed, and this brought about a marked change in the pH of the external medium. Jacobson has found that wheat plants one hundred days old can change the reaction of a culture medium from an original pH of 3·9 to one of 6·3, whereas rice plants brought about the reverse change. With rice plants, a culture solution of initial pH of 5·0 was changed in the course of three days to 3·0. Jacobson attributes these results for the wheat plants to the selective absorption of nitrate ions from potassium nitrate, potassium ions being left, and for rice, to the greater absorption of cation from sulphate, the sulphate anion being left.

THE RELATIVE CONCENTRATION INSIDE AND OUTSIDE THE CELL

Stiles and Kidd* have measured the rate of entry of various salts into slices of carrot and potato. It was found that the carrot answered the purpose better than potato as there is less exosmosis from this tissue. The slices were cut to a diameter of 1·8 cm. and 1·0 mm. in thickness. Experiments were carried out at constant temperature (20° C.) and constant volume of solution (100 c.c.). Forty such discs were used for each experiment. The slices were placed in the given solution and the conductivity measured before and after insertion. To counteract the effects of exosmosis, the slices were first placed in distilled water and the conductivity measured; this value was subsequently subtracted from the final result. The following results were obtained with solutions of sodium, potassium and calcium chloride:

KCl (*N*/5000)	*NaCl* (*N*/5000)	$CaCl_2$ (*N*/5000)
Conductivity fell. In higher concentrations there is a rise in conductivity.	Conductivity fell.	This salt enters more slowly than salts of monovalent metals. Slight losses in conductivity occur.

Stiles and Kidd endeavoured to determine the concentration on the two sides of the cell membrane after complete absorption had occurred. They measured what they termed the "absorption

* *Proc. Roy. Soc.* (*Lond.*), 1919 **90B**, 448, 487.

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ratio," i.e. the ratio of internal concentration to external concentration. Knowing the size of each disc they were able to calculate the volume of cells within the disc, and knowing the amount of salt that had entered they were able to calculate the concentration within the cells. In weak solutions it was found that the internal concentration was greater than the external, whilst in strong the reverse held good. The equation:

$$y = Kc^m,$$

in which c represents the external and y the internal concentra-

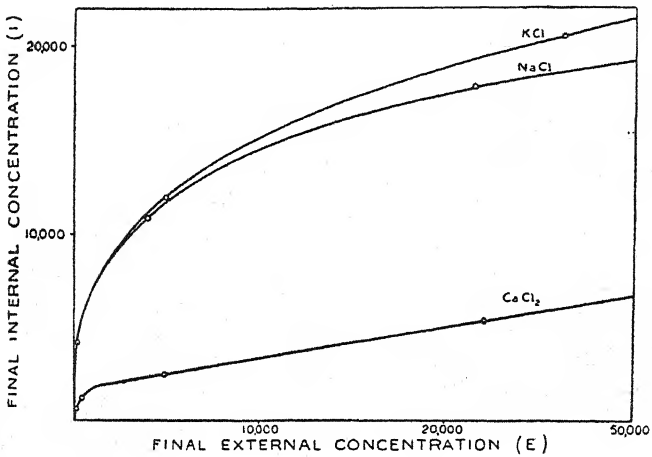

FIG. 8.—Curves showing the relationship between final external concentration for carrot tissue immersed in solutions of potassium, sodium, and calcium chlorides. (After Stiles and Kidd.)

tion and K and m are constants, was found to hold good for the absorption of salts by the cell. Very much the same type of curve was obtained as for adsorption of electrolytes by a colloid (Fig. 8).

Experiments conducted with aluminium sulphate showed a marked rise in the conductivity of the external medium. One of two explanations may be advanced to account for this result. It might have been due to the death of the cells under the toxic action of aluminium, with the result that the plasma-membrane was no longer semi-permeable and allowed the passage of salts from the interior of the tissue. The second and correct explana-

tion was that the rise in conductivity was due to the replacement of aluminium ions by hydrogen ions from the cells of the tissue. It must be borne in mind in this connection that for every aluminium ion that enters the cells, three hydrogen ions will diffuse out into the external medium, and since the hydrogen ions are more mobile, a rise in conductivity was found.

Stiles has shown that when storage tissue is immersed in a solution of a single mineral salt, the ions of the salt are not absorbed necessarily in equivalent amounts, the balance of the ionic changes in the external solution being maintained by the diffusion of ions out from the tissue. Absorption of either ion proceeds fairly rapidly at first, but after some four to five hours the rate of absorption falls off greatly. The absorption of each ion proceeds towards an equilibrium condition, which is not that of the equality of concentration of the ion within and without the tissue, but which is dependent on the concentration. The absorption ratio varies continuously from a fraction of unity to many times unity with decreasing concentration of the salt, and the position of equilibrium is given with fair approximation by the absorption ratio we have already discussed above. The process of absorption is therefore a complex one, and indicates that the absorption of salts by the living cell is not a simple process of diffusion. Two explanations are possible: either the salt combines chemically with some cell constituent, or the salt and its ions are adsorbed at the surface of some cell constituent or constituents. The latter appears to be the more reasonable suggestion. The protoplasm of the living cell is a complex colloidal system, and there is evidence that the material in the cell vacuole is also colloidal in nature so that the plant cell may well act as an adsorbent of inorganic salts.

Steward,* who has carried out an elaborate series of investigations on the absorption of salts by storage tissues, such as potato and carrot, considers that the problem of absorption of salts assumes a complexity which has not been adequately realized by previous investigators. In the past, storage tissues have been much used for absorption experiments on account of simplicity and freedom from complications due to metabolism and growth, which undoubtedly complicate the interpretation of data obtained from actively growing plants.

* *Protoplasma*, 1932, **15**, 29, 497; **16**, 576; **17**, 436; 1933, **18**, 208; *J. Exp. Biol.*, 1934, **11**, 103; *Ann. Bot.*, 1934, **48**, 395; 1936, **50**, 345; *Plant Physiol.*, 1936, **11**, 509.

Steward claims that, in this question of absorption of salts by storage tissues, it is the oxygen-carbon dioxide relations which are of dominant importance and this fact has not been grasped by investigators. Adequate aeration of the tissues is of the utmost importance during the experimental period, and it is also necessary to use relatively large volumes of solution. Steward followed the absorption of potassium bromide by discs cut from potato tubers. He discovered that the bromide is readily absorbed and occurs in true solution in the cell sap, and that the internal concentration far exceeds the external. He showed that the conditions which determine salt accumulation by potato discs also determine the aerobic respiration of the discs. Respiration gives the necessary energy for the performance of work by the cells and is of fundamental importance to salt absorption. For example, it was found that undue mechanical agitation of the tissues led to a fall in salt absorption, and further, that accumulation of bromide by the tissues ceases below a temperature of 5 to 6° C. If salt accumulation is merely an expression of physico-chemical equilibrium, it becomes difficult to understand why it should be affected by different variables which also affect the rate of respiration. Steward is doubtful if the living cell can be regarded as a system in equilibrium, and suggests that equilibrium criteria are only satisfied by dead cells.

There is another point that needs consideration in connection with the use of discs of storage tissue in permeability experiments. In experimental work in which discs of tissue have been used, the tacit assumption has always been made that the behaviour of the cells is uniform throughout the mass of tissue. Consideration will show that the assumption is by no means valid. Steward, who has so strongly advocated the importance of taking account of metabolism in absorption experiments, has shown that it is the cells at the surface of the cut discs that absorb potassium bromide from solution. These cells will naturally be more in contact with oxygen, either dissolved in water or in the gaseous phase, and will therefore respire more actively than cells in the interior. Salt accumulation from this work is dependent upon metabolic phenomena which presumably supply the necessary energy for absorption, and maximum absorption is associated with maximum respiration.

According to Scarth,* who has observed the entry of ions into

* *Amer. J. Bot.*, 1925, **12**, 133.

the cells of Spirogyra by a method depending upon the contraction in length of the chloroplasts, using the long axis and strands of cytoplasm as an index, many divalent and trivalent cations are absorbed at first with great rapidity, but that the rate of absorption slows down to a standstill. When the initial penetration of ions was plotted against external concentration, the curve rose sharply at first, and then flattened out and finally fell away with higher concentrations. Scarth claimed that the penetration of an ion into a cell is determined by two opposing reactions taking place in the interior of the cell—one tending to the absorption of the ion and the other to its exclusion. The latter process takes some time to reach its maximum intensity, and in the meanwhile the former process reaches its maximal activity and then declines. The sensitivity of both reactions is considered to increase with the valency of the ion. Scarth has stated that the absorption reaction, for example, increases with the atomic weight of the ion in any one chemical group, and varies with the solution pressures at the heavy metal end of the series.

With regard to the order of entry, cations have been found to follow the sequence: $K^{\cdot}$, $Ca^{\cdot\cdot}$, $Na^{\cdot}$, $Li^{\cdot}$, $Mg^{\cdot\cdot}$, $Zn^{\cdot\cdot}$ and $Al^{\cdot\cdot\cdot}$. These are the initial values. At a later time the order of entry is: $K^{\cdot}$, $Na^{\cdot}$, $Li^{\cdot}$, $Ca^{\cdot\cdot}$, and $Mg^{\cdot\cdot}$. The order of entry of anions has also been discovered. Initially the following values have been ascertained: SO_4'', NO_3', Cl'; whilst later the order is: NO_3', Cl', SO_4''. The initial entry of ions appears to depend on their relative mobility, but towards the end of the process it appears to depend on some inherent physiological property of the cell, which is not understood at present.

Light.—Various external factors affect the permeability of cells. Lepeschkin* has found that the pulvini, i.e. the swellings at the base of the petiole of *Phaseolus vulgaris*, are more rapidly penetrated by dissolved substances in the light than in the dark. Three experimental methods were employed. In the first, use was made of exosmosis. A hundred pulvini were severed and divided into two sets of fifty and placed in water. One set of fifty was allowed to remain in the dark, and the other exposed to light. At the end of a given time the water was evaporated off and the residue weighed. It was discovered that the pulvini exposed to the light showed the greater permeability, i.e. the greater amount of salt was found. In the second method, the turgor

* *Ber. d. deut. bot. Ges.*, 1911, **29**, 349.

of the cells was taken as a criterion. The tissues were placed in water and the fall in turgor observed. It was ascertained that the turgor fell faster in the light than in darkness. In the third method, plasmolysis was used. The tissues were placed in solutions of cane sugar and potassium nitrate. The greatest amount of plasmolysis was found to take place in the dark, again showing that the cells exposed to light exhibited the greater permeability.

Tröndle* has also carried out some similar work, using the leaves of *Acer platanoides*, *Buxus sempervirens* and other plants. By the plasmolytic method, it was discovered that the greatest amount of plasmolysis occurred in the dark, the permeability of the mesophyll cells being especially increased by illumination.

In this question of the action of light on the permeability of cells, it would appear that it is the amount of light that is the important factor; whether it be strong light for a short time, or weak light for a longer period, the ultimate effect is the same, provided that the amount of light that falls on the cells is the same in each case.

It has been found that if I_1 and I_2 represent the intensities of light and T_1 and T_2 the times during which the light is allowed to fall on the tissues concerned, then the above results can be expressed in the form of the equation:

$$I_1(T_1 - K) = I_2(T_2 - K)$$

K being some constant. This result would seem to suggest that some photo-chemical change proceeds in the cells under the influence of light, and that the amount of this change depends on the amount of light that falls on the cells.

V. H. Blackman and Paine† in their investigations on the pulvinus of *Mimosa pudica* employed a method depending on exosmosis of electrolytes and the simultaneous measurement of the conductivity of the external liquid. The current was found to alter with the rate of exosmosis of electrolytes from the cells of a split petiole. On exposure to light there was a rise in conductivity, while in the dark the conductivity fell. Again this result shows that there is an increase of permeability of the cells in the presence of light.

M. M. Brooks‡ has examined the influence of light on the permeability of Valonia placed under screens which allowed different

* *Jahrb. f. wiss. Bot.*, 1910, **48**, 171; *Viert. d. Nat. Ges.*, Zürich, 1918, **63**, 187.
† *Ann. Bot.*, 1918, **32**, 69.
‡ *Protoplasma*, 1926, **1**, 305.

wave-lengths of light to pass through (300 $\mu\mu$ to 700 $\mu\mu$). It was found that the amount of 2·6-dibromophenolindophenol that penetrated the cell increased with decrease in the wave-length of light. According to Brooks, the penetration of this dye followed a unimolecular reaction and the expression:

$$K = \frac{1}{t} \cdot \log \frac{a}{a - x}$$

could be used to calculate the curves obtained, a being the estimated amount of dye that had penetrated the cells at equilibrium and x the amount of dye at any time t.

Effect of Temperature on Absorption.—With tissues of potato, Stiles and Jørgensen* have ascertained that the relation between absorption of hydrochloric acid, or rather of the hydrogen ions of that acid, and temperature, is practically logarithmic, and the expression representing the absorption rate is approximately given by:

$$\frac{dx}{dt} = K(A - x)$$

where $\frac{dx}{dt}$ is the rate of absorption at any time when x represents the diminishing concentration of acid in the external medium and A represents the original concentration. The absorption of the acid was followed for the temperatures, 0°, 10°, 20° and 30° C. K, in the equation given above, is determined by the temperature. In the table given below are shown values of K calculated for the different temperatures employed:

Temperature	K
0° C.	0·036
10° C.	0·081
20° C.	0·174
30° C.	0·380

From these results, it can be said that for every rise in temperature of 10° C., the rate of absorption of hydrogen ions by potato tuber is approximately increased 2·2 times. Whether this result is to be correlated with any definite chemical reaction, or is merely due to absorption, is difficult to say. From the recent investigations of Steward, metabolism probably plays a large part in the matter and may account for this result. It will be

* *Ann. Bot.*, 1917, **31**, 415.

recalled that Steward found that absorption of potassium bromide by potato tuber ceased below 5° C.

THE ANTAGONISM OF SALTS

It was first shown by Loeb in his studies on the physiological reactions of a number of marine organisms placed in different salt solutions, that solutions of single salts were very much more toxic in their action than solutions composed of two or more salts. Working with the eggs of Fundulus, Loeb was able to show that the development was inhibited if immediately after fertilization they were placed in a solution of sodium chloride having the same concentration as this salt has in sea water. If, however, small quantities of the salt of a divalent metal were added, e.g. salts of calcium, strontium or magnesium, development of the embryo proceeded normally. A number of salts when present together in solution appear to be able to antagonize the toxic effects of each other. This phenomenon is termed *antagonism of salts*, and such solutions are called *balanced solutions*. Sea water is a typically balanced solution.

Osterhout has shown that ordinary water cultures are really balanced solutions. The various salts present are in themselves toxic to the well-being of the plant, but when mixed together in solution they are harmless and appear to antagonize each other's toxic effects. In the case of experiments on the effects of different elements on growth (see Chapter XI), in which one element is deliberately excluded from the water culture, a double result is in reality obtained. In the first place, the plant is deprived of a particular element, and in the second, the physiological balance of the solution is altered at the same time. The alga Cladophora, for example, will live for several days in distilled water, but when placed in weak solutions of potassium nitrate or sodium chloride death is very rapid.

The antagonistic effects of mixed salt solutions on the eggs of Fundulus were found by Loeb only to hold good as long as the surrounding membranes remained intact. If the outer membrane of the egg were damaged in any way antagonistic effects were no longer shown. Loeb therefore concluded that the seat of antagonistic activities must be sought in the cell membrane.

Osterhout has followed the effect of different salts on the growth of the germ tubes of Botrytis* and also wheat seedlings.

* *Bot. Gaz.*, 1906, **42**, 127.

It was found that growth was retarded in single salt solutions, while it went forward normally in solutions of mixed salts; the greatest amount of growth was always found when one of the salts was slightly in excess of half the total concentration.

The mineral solutions of the soil are balanced solutions. It has been found that not all single salt solutions are toxic to the same degree. Of the chlorides of sodium, potassium, and calcium, sodium is the most toxic and calcium the least toxic to wheat seedlings. Mixtures of these three salts antagonize each other's toxic effects. Trelease and Trelease* have tried a wide range of concentrations of single salt solutions on the growth of roots of wheat seedlings and found in all cases that magnesium was the most toxic in its action. The concentration of single salt solutions also markedly affects their toxic action. Thus potassium nitrate in low concentrations was less toxic than calcium nitrate, but in higher concentrations the reverse is true. Temperature also influenced the action of the salt. The greatest variation in the growth of wheat seedlings was found at 30° C. in single salt solutions. It has been shown by Eisenmenger† that the order of toxicity is different for different degrees of toxicity. Thus, for slight retardation of growth of wheat seedlings, the order of the three salts used in this work is KH_2PO_4, $Ca(NO_3)_2$, $MgSO_4$, whereas for severe retardation of growth rate the order is $MgSO_4$, KH_2PO_4, $Ca(NO_3)_2$.

The antagonism of salts has been shown to hold good for the growth of bacteria. Lipman‡ has found in the case of *Bacillus subtilis*, that when peptone was used as a medium and the ammonifying power of the bacteria as a measure of their activity, this activity was reduced in single salt solutions. In the presence of sodium chloride (except a 1/10*M* solution, in which a slight increase was shown), potassium chloride and calcium chloride, the amount of ammonia was decreased. Of these three salts, calcium chloride was found to be the most toxic in its action. Solutions composed of a mixture of sodium chloride and magnesium chloride showed marked antagonism. No antagonism, however, was shown with a mixture of magnesium chloride and calcium chloride.

Szücs§ has shown that the geotropic responses of roots were

* *Bot. Gaz.*, 1925, **80**, 74; *Bull. Torrey Bot. Club*, 1926, **53**, 137, 605.
† *Bull. Torrey Bot. Club*, 1928, **55**, 261.
‡ *Bot. Gaz.*, 1909, **48**, 105. § *Jahrb. f. wiss. Bot.*, 1913, **52**, 85; 269.

altered in the presence of certain toxic salts. The roots were laid in a horizontal position and treated with solutions of various salts; the standard taken was when 70 per cent refused to answer to the call of gravity. Szücs was able to ascertain that copper sulphate was the most toxic of all the compounds employed, even in very dilute solution. Aluminium chloride was only weakly poisonous. If the two salts, aluminium chloride and copper sulphate were mixed they formed a balanced solution and antagonism was shown, the roots responding to gravity in the normal way.

Szücs* also found that antagonism exists between aniline dyes and electrolytes. With methyl violet in the presence of potassium nitrate, entrance of the dye into the cells of Spirogyra was greatly hindered. Similar results were obtained with calcium and aluminium nitrate. The extent of the antagonism was found to depend on the concentration and valency of the salt employed. The higher the valency the greater the antagonistic effect. It was discovered by Szücs that the antagonism between dye and potassium nitrate could be expressed by the equation:

$$T = kC^{1/n}$$

where C is the concentration of the electrolyte, T is the time for the dye to enter the cell and k and n are constants. This expression was found to hold only approximately for aluminium and calcium nitrate. Szücs concluded from the nature of this equation that the intake of dye into a cell is dependent on the adsorption of electrolytes by the protoplasm. Endler† working with *Elodea densa*, *Ulva lactuca* and *Nitophyllum punctatum* arrived at a different conclusion from Szücs. From his experimental results he observed that the rate of entry of dye was very much increased in the presence of electrolytes. It must be remembered, however, in this connection, that the dyes and electrolytes used by Endler were different from Szücs, and further, that Endler was more concerned with the absorption of dye at the equilibrium point.

Irwin‡ has suggested a method whereby it is possible to separate the action of the electrolyte on the protoplasm from that of the dye. The tissue is placed for a given time in the salt solution, and this is followed by immersion in a solution of the dye which contains no salt. The penetration of the dye into such cells is then compared with the controls (cells which have not been previously

* *Sitzungsber. kais. Akad. Wiss. in Wein, Mat-nat. Kl.*, 1910, **119**, 737.
† *Biochem. Zeit.*, 1912, **42**, 440; 1915, **45**, 359.
‡ *Proc. Soc. Exp. Biol. and Med.*, 1926, **24**, 54; *J. Gen. Physiol.*, 1926, **10**, 271, 425.

exposed to the action of the salt solution). From these observations it is possible to arrive at the effect of the electrolyte on the protoplasm since there is no error introduced by the salt adhering to the surface of the cell wall, nor does salt diffuse out of the cell. In the second place, tissue previously exposed to distilled water or salt solution is immersed in the dye solution containing dissolved electrolyte, and the rate of penetration of dye is compared with controls (cells which have been previously exposed to distilled water and then placed in a dye solution containing no salt). In the case of cresyl blue, the penetration of the dye was delayed when the tissue was exposed to the action of monovalent salts, e.g. sodium chloride, before they were placed in the dye solution. It is possible that this inhibiting action might have been due to the effect of the salt on the protoplasm. When a monovalent salt was mixed with the salt of a divalent metal, e.g. sodium chloride and magnesium chloride, antagonism was shown and the rate of entry of the dye was not affected. The previous immersion of the tissue in solutions of divalent or trivalent ions produced no effect on the entrance of the dye. These results of Irwin in a large measure reconcile the conflicting reports of Szücs and Endler.

Ringer's Solution.—Ringer's solution consists of a mixture of sodium chloride (0·67 per cent), potassium chloride (0·01 per cent), calcium chloride (0·02 per cent), and sodium bicarbonate (0·02 per cent). When the heart of a frog is perfused with this mixture it will continue to beat for a considerable time. If, however, a solution of sodium chloride alone be used, the beats cease at once. On the addition of the other salts of this solution the beats may be made to continue. Ringer's mixture is a typical case of a balanced solution.

It was originally shown by Ringer that the salts of the elements caesium and rubidium, which occur in the same group of the periodic table as potassium, were able to replace this element in his mixture. Of all the elements composing this solution, potassium is the only one which exhibits feeble radioactivity and expels β-rays, and is some thousands of times less radioactive than uranium, while radium is 10^9 times as active. Rubidium is even less radioactive than potassium, and radioactivity has been denied by many for caesium. It was shown by Zwaardemarker and Feenstra* that a frog's heart will beat equally well in a Ringer's

* *Proc. Akad. Wetensch. Amsterdam*, 1916, **19**, 99, 341, 633; 1917, **20**, 773; 1918, **20**, 768; *Pflüger's Arch.*, 1918, **173**, 28; *Arch. Néerland Physiol.*, 1917, **1**, 748.

mixture which contains, instead of potassium chloride, a calculated quantity of uranium nitrate, thorium nitrate, radium bromide or niton, equivalent in radioactivity to the amount of potassium in normal Ringer's solution. In other words, a frog's heart which has stopped beating in a potassium-free solution will recommence to beat when an equivalent quantity of a radioactive element is added to the fluid. They also found that a heart could be made to beat when it was exposed to β-radiation from mesothorium or radium at a distance of 1·2 cm. These original experiments were carried out in winter, and it was found that in summer smaller quantities are sufficient and the reduction in amount may be brought about by the addition of eosin or fluorescein to the solution. In any case, the reduction in amount appears to be due to the better adsorption of the radioactive element by the endothelium of the frog in summer. The following paradox is explained by the assumption that electrically charged particles are adsorbed. It was found, for example, that the various methods of keeping the heart pulsating or restoring its beat when it had stopped may be arranged in two classes:

Group I	*Group II*
Potassium	Uranium
Rubidium	Thorium
Caesium	Radium
β-Radiation	Niton

A heart beating under the action of one of the elements in the above two classes will continue to beat when it is supplied with another member of the *same* group, but ceases if supplied with the element from the other group. Thus, either β-radiation-Ringer or radium-Ringer will restart a heart stopped by the use of a Ringer solution free from a radioactive element. In the case of β-radiation-Ringer solution, the heart will continue to beat if β-radiation be replaced by either caesium or rubidium, but stops at once if it be supplied with thorium or uranium or any other member of the second group. Before this "switching-over" from one group to the other can take place, the heart must be well washed with a solution free from radioactive elements. According to physicists, we are dealing in Group I with elements which are expelling negatively charged particles, whereas the elements in Group II are discharging positive α-particles. In radium both kinds of particles are being discharged, but the α-particles predominate. The adsorption of either kind of particle

gives to the heart the necessary electric charge, but in a mixture of the two groups the two different kinds of charge will neutralize each other and the necessary electrical condition cannot be maintained.

An interesting botanical analogy has been discovered by Zwaardemarker in this connection. The centres of plates containing luminous bacteria were exposed to mesothorium, which expels α-particles and β-radiation from polonium, and the cultures were subsequently photographed by their own light, when in both cases it was found the centres were black owing to the local death of the bacteria in this area where the two types of ray had neutralized each other.

THE NATURE OF ANTAGONISM

The nature of antagonism is obscure. It is known that mixtures of two salts enter a cell very much more slowly than single salts. Loeb, therefore, considered the whole matter of antagonism to be due to the keeping out of salts from the cell. Osterhout has shown this very well in Spirogyra. The alga was immersed in a solution of sodium chloride (strength 0·379 molar). A solution of this concentration just brought about plasmolysis of the cells. In a solution of sodium chloride of concentration $0 \cdot 375M$, there was no plasmolysis. Similarly Osterhout found that solutions of calcium chloride of strength $0 \cdot 2M$ and $0 \cdot 195M$ just did and just did not bring about plasmolysis. When the two solutions of strength $0 \cdot 375M$ NaCl and $0 \cdot 195M$ $CaCl_2$, i.e. the non-plasmolysing solutions, were given together, plasmolysis occurred, showing that there had been no entry into the cells. The question naturally arises, Why should a single salt be more toxic than a mixture? The mere slowing down of the rate of entry will not explain the matter, for to whatever extent the rate of penetration may be slowed down, the final result will be the same. The most probable explanation is that the protoplasmic proteins adsorb salts on their surface in certain definite proportions and unless these salts are adsorbed in these definite ratios the proteins are no longer in an active state. In pure water the salts are slowly washed out, while in single salt solutions the salt tends to replace the adsorbed mixture and the injurious effect is produced by the alteration in the correct proportions which were originally present.

Osterhout has brought forward a so-called "dynamical"

explanation of antagonism based on experiments with Laminaria. He assumed that two processes are involved, one tending to produce a fall in resistance of the tissues and the other a rise, and considers that the two processes may be represented by the following scheme:

$$A \rightarrow M \rightarrow B$$

in which A breaks down to give an intermediate substance M, which by further decomposition furnishes B. At the same time he assumed that the electrical resistance of the protoplasm is due to M and that the resistance of the tissue is proportional to M plus some constant equal to the resistance of the tissue when dead. Under natural conditions, such as the life of Laminaria in the sea, it is supposed that the velocity-constants of these reactions remain constant, but when transferred to a single salt solution, or to a mixture of two salts, the velocity-constants of the reaction $A \rightarrow M$, and $M \rightarrow B$ become in some way markedly altered. If the reaction $A \rightarrow M$ should proceed at a greater rate than the reaction $M \rightarrow B$, M will accumulate, and the resistance will gradually rise until the supply of A is exhausted, when M will be more and more slowly formed, and finally it will give rise to B at a greater rate than it is formed from A and there will be a fall in the resistance of the tissue. This theory assumes a great deal more than is warranted by the facts and ignores the highly complex nature of the system involved.

Raber* has brought forward an explanation of antagonism based on the electrical charges of salts and protoplasm. It is probable that the charge on the surface of protoplasm is negative. The cell sap, on the other hand, is generally slightly acid, forming with the protoplasm an electrical double layer. The effect of salts on the protoplasm will depend on the nature of the charges on their individual ions. In a divalent salt such as calcium chloride, two positive charges are concentrated on one ion, the cation, whereas the two negative charges are divided between the two anions. The dominant effect of such a salt will depend on the cation with its two charges. In the case of such a salt as sodium sulphate, Na_2SO_4, it is the anion which carries two negative charges and will produce the dominant effect. Raber terms calcium chloride a "positive" salt and sodium sulphate a "negative" salt. When protoplasm first comes into contact with

* *Bot. Gaz.*, 1923, **75**, 298.

a positive salt, the charged particles on the surface will "tighten" or become more drawn together on account of the positive charge on the cation, but later as more salt diffuses in, all the particles on the surface of protoplasm will become positively charged and repel each other, so that the first effect of the "positive" salt will be to cause a decrease, and later an increase, in permeability. With a "negative" salt, on the other hand, there will be a continuous increase in permeability from the very outset. For reasons which cannot be entered into here, a monovalent salt such as sodium or potassium chloride behaves like a "negative" salt when placed in contact with a negative colloid, so that, according to Raber, the toxic effects of single salts on protoplasm is a result of the abnormal changes they bring about on the protoplasmic membrane by virtue of the electrical charges which they possess. Antagonism results because these effects are opposite, and when the salts are mixed together in the proper proportions the electrical charges are neutralized and toxicity disappears in a balanced solution since the salts and their ions are electrically balanced. Raber considers that it is not a mere coincidence that of the six elements which a plant removes from the soil in any great amount, $K^{\cdot}$, $Ca^{\cdot\cdot}$, $Mg^{\cdot\cdot}$, S'', P''' and N', three occur in the anion with six negative charges, whereas three in the cation have five positive charges. He claims that it is this property which allows of a "three-salt" solution in which the electrical charges are evenly balanced.

MECHANISM OF ABSORPTION

The study of the mechanism of entry of salts into the living cell has led to the production of endless theories of varying worth. It seems to be a point of honour among investigators on cellular permeability to increase the already large number of theories by some new scheme whenever the opportunity will allow. Osterhout* has now advanced the view that the penetration of living protoplasm is confined to undissociated molecules and that ions are unable to enter. He has derived this view mainly from work on Valonia and the use of ammonium chloride. Support for the theory is also based on results obtained by the use of "models" which are considered to imitate the living cell. The use of such "models," however, is of very doubtful value in the study of such a complex problem as cell permeability, and little

* *Biol. Rev.*, 1931, **6**, 369; *Engeb. Phys. und exp. Pharm.*, 1933, **35**, 967.

or no reliance can be placed on data obtained in this way. M. M. Brooks* and others have been unable to find any support for Osterhout's view. Hoagland,† for example, found in the case of Nitella that the cells are able to take up bromine from potassium bromide without damage to themselves, and that the final concentration of the bromine within the cells might in some cases be as high as sixty times that of the external medium. Furthermore, this process was only able to proceed in the light, and no or very little absorption of bromine occurred in the dark, and the temperature coefficient was found to be comparable to that of a chemical reaction rather than that of a simple diffusion process. In view of the very dilute solutions of potassium bromide that were used in this work, Hoagland maintains that ions and not undissociated molecules enter the cell.

Some further experiments by Hoagland, Hibbard and Davis† bear upon this problem of absorption of salts by the living cell. They found that when they immersed equal masses of healthy cells in slightly acid solutions of potassium chloride (0·001*M*), and allowed part to remain in the dark and the remainder to be illuminated for varying periods of time, such as three, five and eight hours, there is a very definite correlation between absorption and illumination. Only slight absorption occurred in the absence of light, or if the period of illumination were short. Hoagland, Hibbard and Davis therefore considered that the energy derived from light is directly concerned with the penetration of substances into the cell. In the case of roots, the majority of which live in complete darkness, the energy for the purpose of absorption is thought to be derived by the breakdown of carbohydrate in respiration.

It is essential to realize that plants, and probably living cells in general, have the power to bring about movements of solutes against a concentration gradient. G. E. Briggs‡ has also brought forward theories regarding the absorption of salts by the cell, but the experimental data are meagre and those interested should consult the original papers.

Hoagland was the first to advance the view that energy obtained from metabolism was a necessary factor in salt absorption, and this view has been much elaborated by Steward.§

* *Amer. J. Physiol.*, 1926, **76**, 116.
† *J. Gen. Physiol.*, **10**, 121; *New Phyt.*, 1925, **24**, 99; *Plant Physiol.*, 1936, **11**, 471.
‡ *Proc. Roy. Soc. (Lond.)*, 1930, **107B**, 248; *Ann. Bot.*, 1932, **46**, 301.
§ *J. Exp. Biol.*, 1934, **11**, 103; *Ann. Bot.*, 1934, **48**, 395; 1936, **50**, 345; *Plant Physiol.*, 1936, **11**, 509.

Some of the work of the latter author has already been considered above. Hoagland and Steward consider that the metabolic activities of the cell have been overlooked and disregarded in this connection. Certainly this criticism is well justified, and it is odd that an important point of this kind should have escaped notice. From the point of view of Steward, absorption of mineral elements is determined by and related to the capacity of the cell for further growth and metabolism, and it is the aerobic phase of metabolism that is closely concerned with salt accumulation. Granted the capacity to grow, capacity for accumulation follows.

CHAPTER V

TRANSPIRATION

THE loss of water from the aerial parts of plants is called transpiration. The main mass of transpiration takes place from leaves, but twigs and stems also transpire to a limited extent. It was shown towards the close of the eighteenth century by Stephen Hales, that the water lost by a sunflower plant with a total leaf area of 9 square metres was 1 pint during a dry summer day. Von Höhnel has calculated that an average birch tree with approximately 200,000 leaves, lost from 300 to 400 kg. of water in a single day, and in a birch wood of $2\frac{1}{2}$ acres containing 400 to 600 such trees the total water loss in six months would be 2·4 to 3·5 million kg. Haberlandt has calculated that in a $2\frac{1}{2}$-acre field of oats, 2,277,760 kg. of water were lost in a growing season. This approximation is probably too high. Haberlandt failed to take into account the effect of plant on plant, and the fact that under field conditions a plant surrounded by others will not transpire to the same extent as a solitary plant in the open. More recently Balls has estimated that the loss of water from an Egyptian cotton crop by transpiration is 50 tons per acre per day, or 3 pints per plant. It will be seen from the various estimations given above that the loss of water from the living plant through transpiration is very great.

METHODS OF ESTIMATING TRANSPIRATION

Several methods are available for the quantitative estimation of transpiration, and they all suffer from various disadvantages.

(1) *Gravimetric Method.*—Of all the methods that have been described for the estimation of transpiration, the so-called gravimetric method is perhaps the best. In this method, the plant is weighed from time to time, and the loss of water directly determined. In actual practice, a potted plant is placed in an aluminium sheath, and the surface of the soil in the pot covered with wax or mackintosh sheeting, so that direct evaporation of water from the soil is prevented. The pot and plant are weighed at given intervals and the loss in weight recorded.

A number of modifications have been introduced into this

method and special balances have been devised to allow of automatic weighing. V. H. Blackman and Paine* have described a highly ingenious system in which drops of water of constant size are delivered from an aspirator into the plant container through a small glass tube. The plant and container are weighed on an

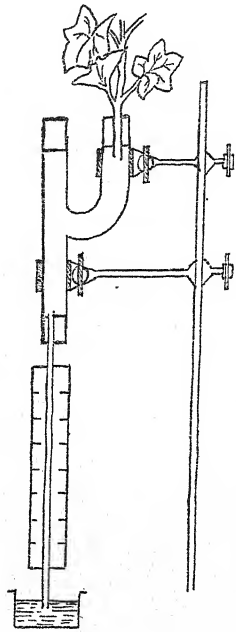

FIG. 9.—Potometer. This apparatus measures the relative rates of water absorption by a cut shoot under different external conditions. The glass apparatus is completely filled with water and a bubble of air is introduced into the lower tube and the rate of movement of this bubble is measured on the scale.

automatic balance, and a metal tube or drain is pushed backwards and forwards by means of two solenoids and allows of drops of water entering the pot through the glass tube to counterbalance the loss of water from transpiration. The results are recorded on a revolving drum with a magnetic pen.

The gravimetric method of estimating the rate of transpiration has the inherent defect that under the experimental conditions the proper aeration of the soil is interfered with and the circulation of oxygen prevented. Maximov, however, considers that this

* *Ann. Bot.*, 1914, **28**, 109.

disadvantage has been overestimated and that the sealing of the plant containers does not materially inhibit the proper functioning of the root system. L. J. Briggs and Shantz,* in the course of their numerous experiments on transpiration and soil moisture, sucked air through their sealed plant containers by means of an aspirator and thus obviated the difficulty of improper aeration of the soil.

It is necessary to remember in experiments on transpiration in which the gravimetric method is used, that the plants should not be directly removed from the soil and transferred to pots. It is practically impossible to remove the root system in its entirety, and the roots themselves may become injured in the process, with the result that a distorted picture of the transpiration of the plant is obtained.

(2) *Potometer.*—The potometer, of which there are numerous types, has also been used to measure the rate of transpiration. Fig. 9 illustrates one type that has been used for this purpose. The underlying assumption in this method is that the rate of water absorption and rate of transpiration is exactly equal. This is not the case. In 1889 Eberdt showed that the relation between transpiration and water absorption was not exact and that the one may be in excess of the other as the following figures show:

Time	*Water Absorption Grammes*	*Transpiration Grammes*	*Difference Grammes*
8.45 to 11.45 a.m.	4·95	5·53	− 0·58
11.45 to 3.0 p.m.	5·50	7·40	− 1·90
3.0 to 7.15 p.m.	6·45	5·58	+ 0·87

It will be seen that in the earlier part of the day the amount of transpiration was in excess of water absorption, whereas in the latter part of the day the reverse condition held good. A further objection to the use of the potometer for transpiration determinations lies in the fact that it is usual to employ cut shoots rather than whole plants. In general terms, provided that the environmental factors remain undisturbed, the transpiration rate of cut shoots shows either an increase or decrease on the initial rate and rarely remains constant. If cut shoots must be employed for transpiration determinations, it is better to cut the shoots under water in the evening and allow them to recover during the night.

* *Bot. Gaz.*, 1912, **53**, 20, 229.

(3) *Cobalt Chloride Paper Method.*—Paper soaked in a solution of cobalt chloride has been much used for transpiration experiments. The method was originally devised in 1894 by Stahl, who carried out a number of qualitative experiments with it.

The method takes advantage of the fact that anhydrous cobalt chloride is blue in colour, whereas the hydrated salt is a pale pink. For experimental purposes, filter paper is soaked in a 3 or 5 per cent solution of the salt and dried in an oven. It is preserved in a desiccator over calcium chloride. For determining the transpiration rate, the dried cobalt chloride paper is placed over the leaf surface and covered with a piece of glass to prevent changes due to atmospheric moisture and the time is observed for the paper to turn from blue to pink.

As originally devised by Stahl the method was extremely crude and could not be used for exact work. A number of improvements have been introduced since. Bakke,* for example, used a standard water surface for purposes of comparison, and compared the times taken to produce a colour change on the standard water surface and also on the surface of the leaf. He termed the ratio water surface (N) to leaf surface (N′), the *foliar transpiring power* of the plant. This value gives a measure of the resistance of the leaf to the loss of water compared with a standard water surface.

Bakke attempted with this improved cobalt chloride method to give more or less quantitative definitions for various types of plants, e.g. xerophytes, mesophytes and hydrophytes. Xerophytes, for example, were considered to give the value 0·3 for the N/N′ ratio, while mesophytes gave the value 0·7. This attempt to give a strict quantitative definition to these various classes of plants proved to be without foundation. Well-defined mesophytes and xerophytes have been found to give values which do not conform to the ratios predicted for them.

Livingston and Shreve† have still further improved the cobalt chloride method by employing definite colour standards. Two permanent colour standards were used, one just slightly less intense than the colour of dry cobalt chloride paper, whilst the other was much less intense, but still clearly blue. The colour standards were prepared by immersing filter paper impregnated

* *J. Ecol.*, 1914, **2**, 145.

† *Plant World*, 1916, **19**, 287. See also in this connection, Henderson, *Ann. Bot.*, 1639 **50**, 321.

with ferric chloride in a solution of potassium ferrocyanide, the intensity of colour of the Prussian Blue precipitated in this way could be varied by varying the concentrations of ferric chloride and potassium ferrocyanide. Small strips of paper, with the colour standards at either end and impregnated with cobalt chloride in the centre, are placed on the leaf surface and covered with a piece of glass, and the time for the cobalt chloride to change in colour from the more intense to the less intense blue is taken. The time is also noted for a similar change in colour to take place on a standard water surface. From the observed rates of colour change, the ratio of evaporation from a water surface to a leaf surface can be calculated. This ratio was termed the *index of transpiring power* by Livingston and is related to another ratio introduced by him, *relative transpiration*, which will be discussed in detail later.

The cobalt chloride method, like the other methods of determining the rate of transpiration which have been described above, is by no means ideal for its purpose. It has the great disadvantage of placing the leaf under unnatural conditions. Light, for example, is excluded from the leaf surface covered by the paper, and stomatal apertures will be affected (see below), and the area of the leaf under the paper will on this account tend to transpire at less than the normal rate.

(4) *Other Methods of Estimating Transpiration.*—Calcium chloride or phosphorus pentoxide may be used for determining the rate of transpiration. The plant together with a weighed amount of calcium chloride or phosphorus pentoxide contained in a small vessel is placed under a bell-jar. At the end of a given time the chloride or oxide is weighed again. Any increase in weight will give the amount of water transpired. The chief disadvantage of this method is that the plant is exposed to extremely dry conditions, and this will tend to raise the rate of transpiration. Freeman* has obviated this difficulty by drawing a known amount of air at a definite velocity through a vessel containing the experimental shoot or leaf, and absorbing the water vapour in two U-tubes containing phosphorus pentoxide. A similar experiment is also set up by drawing air through two U-tubes filled with phosphorus pentoxide, but with no plant present, to determine the amount of water vapour in the atmosphere. The difference between these values will give the amount of water transpired

* *Bot. Gaz.*, 1908, **46**, 118.

in a given time. The chief disadvantage of this method is that drops of water tend to condense on the walls of the plant container and are difficult to remove.

STOMATAL AND CUTICULAR TRANSPIRATION

Von Höhnel divided transpiration into two classes, *stomatal* and *cuticular*. Stomatal transpiration is the amount of water loss through the stomata, whilst cuticular transpiration is the amount lost through the cuticle. It has been found that even the thickest cuticles transpire to a certain extent. In ordinary mesophytes the main mass of transpiration takes place through the stomata. In some plants, cuticular and stomatal transpiration is approximately equal. The Jamaican Rain Forests furnish an example of plants in which cuticular is greater than stomatal transpiration. Here the cuticular transpiration represents about 58 per cent of the total water loss of the leaves.

For purposes of distinguishing between cuticular and stomatal transpiration, the cobalt chloride method may be used. Another method is to vaseline the upper and lower surfaces respectively of a series of leaves. Ficus leaves are much used for this purpose, and the leaves are then weighed at intervals. In this method the leaves are severed from the plant and it is therefore necessary to prevent water loss from the petioles by encasing the latter in tightly fitting rubber tubing which is then bound up with thin copper wire. The method originally devised by Garreau can also be employed. Small bell-jars are cemented on the upper and lower surface of a leaf. Each bell-jar contains a weighed amount of calcium chloride and any increase in weight of the chloride at the end of the experimental time will show the amount of water lost from the two surfaces of the leaf. Glass tubes sealed with oil are inserted into each bell-jar to serve as manometers. The chief disadvantage of the method lies in the fact that the leaf is placed in an abnormally dry atmosphere which will tend to raise the rate of transpiration. Nevertheless, the method serves its purpose well enough as a comparative one.

It has already been seen that in the majority of land plants, the main mass of transpiration takes place through the stomata. The total area of the stomata to the total area of the rest of the leaf surface is on the average not more than 1 to 2 per cent. As far back as 1861 Unger showed that the amount of water

evaporated from a free water surface was some 2·8 to 13·8 times as much as that lost from an equal area of leaf surface. If we take the lower value of 2·8, this would represent that 35 sq. cm. of a free water surface is able to evaporate as much water as 100 sq. cm. of leaf surface. Since in the majority of leaves the total area of the stomata is only 1 to 2 per cent of the total area of the leaf, it follows that the combined area of the stomata is ten times as efficient for the evaporation of water as a free water surface of equal area.

The physics of diffusion through perforated septa was investigated by Horace Brown and Escombe.* Their experiments were mainly concerned with the diffusion of carbon dioxide through stomatal pores, but they also considered their results in connection with transpiration. The method employed was essentially simple in nature. A nickel cover was sealed to a wide glass tube containing a layer of sodium hydroxide solution at the bottom to absorb carbon dioxide. The nickel cover was pierced with a pore of the size to be investigated and after a given time the amount of carbon dioxide absorbed by the sodium hydroxide solution was estimated. It was found that the rate of diffusion was proportional to the diameter and not to the area of the pore. In other words, the smaller the pore the greater will be the diffusion of gas per unit area. This result applies equally to the diffusion outwards of a gas through a pore, i.e. it applies as well for the diffusion into the leaf of carbon dioxide as for the diffusion of water vapour out of the leaf in transpiration. Brown and Escombe pictured the diffusion of a gas through a pore as a welling out in a series of shells of diffusion, and further, that these shells would be nearly elliptical near the mouth of the pore and would become hemispherical at a short distance from the orifice and that over the surface of each shell the density of the gas would be equal. The diffusion of a gas in such a problem as this in reality takes place from a cylinder, since the stomatal pore possesses depth as well as area and may be diagrammatically represented as in Fig. 10.

If the rate of diffusion under static conditions be considered, the general expression for the amount of diffusion in a given time is:

$$Q = K\frac{\rho - \rho_1}{L} \cdot A \cdot t$$

* *Phil. Trans. Roy. Soc.* (*Lond.*), 1900, **193B**, 223.

in which Q is the amount of carbon dioxide flowing down the cylinder towards an absorptive surface, A is the area of cross-section, L is the length of such a cylinder, t is the time and ρ and ρ_1 represent the density or partial pressure of carbon dioxide in the outer atmosphere and at the surface of the absorbent respectively, and K is the diffusion constant of carbon dioxide in air in C.G.S. units. In the case of the diffusion *out* of a gas from such a pore, the values of ρ and ρ_1 would have to be

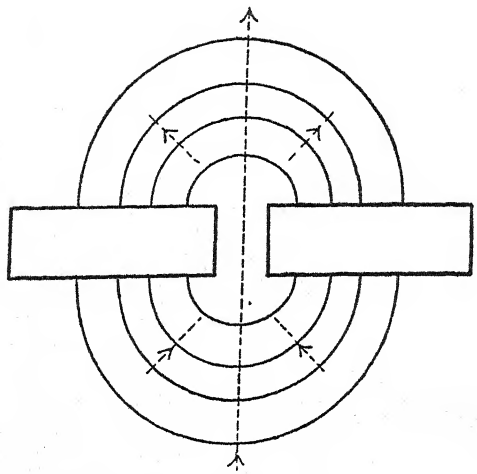

FIG. 10.—Diagrammatic representation of diffusion shells inside and outside a pore in a septum. The direction of flow of the gas is indicated by the arrows. (From Skene, *Biology of Flowering Plants.*)

reversed. From this formula it follows that it is the radius and not the area that determines the relation of the size of the pore to the diffusion rate.

If we consider the flow of a gas through a number of perforations, the problem becomes more complicated by the mutual interference of one aperture with another. Brown and Escombe considered that if the apertures were separated by a distance equal to ten times their diameter, this complication would not enter into the problem and each aperture would act independently of its neighbours. As a general rule, the stomata are separated from each other by a distance less than ten times their diameters. Again, the pore of a stoma is not completely circular but elliptical in shape. Both these considerations introduce further difficulties

into the problem. The equation formulated by Brown and Escombe for the total diffusion through all the stomata from 1 sq. cm. of leaf surface per hour was:

$$Q = n \times K \frac{\pi R^2(\rho_1 - \rho_0) \times 3{,}600}{L + \frac{\pi R}{2}}$$

where n = the total number of stomata per sq. cm. of leaf surface, R is the radius of the pore and 3,600 brings the results from seconds to hours.

Using this expression Brown and Escombe calculated that the diffusion of water vapour from the leaf of *Helianthus annuus* in wind at a temperature of 20° C. and with a fall in vapour pressure from saturation (0·02 atmospheres) inside the leaf to one-quarter of that amount in the external atmosphere should take place at a rate of 0·1730 gm. of water per hour per sq. cm. The maximum rate obtained experimentally was 0·0276 gm. per sq. cm. per hour. They therefore naturally concluded that maximal transpiration can take place through the stomata, although they offered no explanation as to why the observed and theoretical figures differed so markedly. As Renner* has pointed out, the value calculated by Brown and Escombe is three times the rate of evaporation from a free water surface, which is of course absurd. He therefore assumed that a second series of shells of diffusion existed over the leaf as a whole and has amended Brown and Escombe's original equation to:

$$Q = K(\rho_1 - \rho_0) \frac{2\pi R^2}{\frac{\pi R}{4} + \frac{\pi R^2}{n\pi r^2}\left(L + \frac{\pi r}{2}\right)}$$

where R is the radius of a circle equal in area to the leaf and r is the radius of the pore. On Brown and Escombe's formula, the effect of wind on transpiration is not very great, whereas with Renner's modification and taking cuticular transpiration into account, the effect shown is very much greater.

Jeffries† has called to question some of the results obtained by Brown and Escombe and Renner. He considers it an error to

* *Flora*, 1901, **100**, 451.
† *Phil. Mag. J. Sci.*, 1918, **35**, 270.

assume that the presence of other stomata does not cause interference with the absorption of carbon dioxide when their distance apart is equal to ten times their diameter. He concluded that if there be more than 600 stomata per sq. cm. of leaf surface, the rates of absorption and evaporation will be greatly hindered in the presence of other stomata. Jeffries considered that as long as the expression n^2al is less than unity (where n represents the number of stomata per sq. cm. of leaf surface, a is the radius of each stoma and l is the order of linear dimensions of the leaf), each stoma will act independently of its neighbours. Taking a concrete example, in a leaf 3 cm. long with 33,000 stomata per sq. cm. of leaf surface (this is the value for sunflower), each stoma will act independently of its neighbours when the radius of the stomatal pore is less than 10^{-5} cm. In *Helianthus annuus*, the aperture of an individual stoma is 10^{-3} cm. Jeffries therefore arrived at the conclusion that the stomata must close to one-fiftieth of their full diameter before they are quite independent and free from the zone of action of their neighbours. When the expression n^2al is greater than unity, the rate of absorption will be the same as if the whole leaf were an absorbing surface, and in such circumstances the diameter law of Brown and Escombe will not apply. Nevertheless, it must be remembered that the work of Brown and Escombe, and of Renner, has clearly established the fact that the stomata are, for all practical purposes, alone concerned with the process of diffusion into and out of the leaf, and that the efficiency of this system is more than sufficient to supply the full needs of the plant.

THE FACTORS INFLUENCING TRANSPIRATION RATE

As in the case of other physiological processes of the living plant, such as photosynthesis and respiration, internal and external factors play an important part in influencing the rate of transpiration. The chief external factors are (1) humidity of the atmosphere, (2) wind, (3) temperature, (4) barometric pressure and (5) light. Two important internal factors are (1) stomata and (2) the water-content of the mesophyll tissue.

Humidity of the Atmosphere.—If the leaf be surrounded by an atmosphere saturated with water vapour, there can be no gradient of water concentration set up between the intercellular spaces of the leaf and the outside atmosphere. In consequence, no

evaporation can take place, and the rate of transpiration will be reduced. The water deficit of the air is the main point in such a question, and temperature is an important factor in this connection. A rise in temperature will increase the water deficit, whereas a fall will bring about a decrease. There should be a simple relationship between the rate of transpiration and the moisture present in the atmosphere, if transpiration is simply a process of physical evaporation from the wet walls of the mesophyll cells of the leaf.

The first investigation made on this question was by Francis Darwin,* who measured the rate of transpiration and humidity of the air at constant temperature. A linear relationship was discovered between rate of transpiration and humidity of the atmosphere, a straight line graph being obtained. When produced backwards, however, the line did not cut the axis at the 100 per cent saturation point, but at 105 per cent saturation point. The explanation was found to lie in the fact that the living plant respires in the course of its metabolic activities, and on this account the temperature in the immediate vicinity of the mesophyll tissues will be higher than that of the surrounding air. Under such conditions, the air surrounding the mesophyll cells will not be saturated when the external air is in this condition. Thus the value 105 and not 100 per cent was obtained. From the result of this experiment, Darwin was able to calculate the temperature of the mesophyll cells compared with the surrounding air. The experimental work was carried out at a temperature of 16° C., and the vapour pressure corresponding to this temperature is 13·51. This value plus 5 per cent gives a vapour pressure of 14·2. The temperature corresponding to this is 16·8° C. Thus the temperature of the mesophyll tissues was 0·8° C. higher than the surrounding air. Henderson† has confirmed Darwin's observations. Using *Hedera Helix*, he found that his curve did not cut the axis as far back as the one obtained by Darwin, and that the temperature of the mesophyll cells was only 0·4° C. and not 0·8° C. higher than that of the surrounding air. According to Henderson, humidity changes affect the rate of transpiration for higher humidity values as though the surface of the cells were behaving like a damp surface in a purely physical way, for the graphs were found to follow, within narrow limits, the expression

* *Proc. Roy. Soc.* (*Lond.*), 1914, **87B**, 281.
† *Ann. Bot.*, 1926, **40**, 507.

for change of rate of water loss with change in humidity, i.e. the equation:

$$E_1 = E\left(\frac{S_{T_1} - \frac{y}{100} \cdot S_{t_1}}{S_T - \frac{x}{100} \cdot S_t}\right)$$

was found to apply in such circumstances, where E is the evaporation rate, S_T is the saturation vapour pressure of air at leaf temperature $T°$, $\frac{x}{100} \cdot S_t$ is the percentage of saturation water vapour at temperature $t°$, E_1 is the rate of evaporation when S_{T_1} is the saturation vapour pressure of the air at leaf temperature $T°_1$ and $\frac{y}{100} \cdot S_t$ is the percentage of saturation of water vapour at air temperature $t°_1$.

Wind.—The effect of wind is to increase the rate of transpiration, since it will remove the layers of saturated or partially saturated air over the leaf surface.

Temperature.—Temperature does not play a direct part in influencing the rate of transpiration from a leaf, but the rate is indirectly influenced by the effect of temperature on the water deficit of the air. Thus a rise in temperature will raise the water deficit and increase the transpiration rate, whilst a fall in temperature will lower the water deficit and decrease the rate.

It is possible to express wind, temperature and humidity in terms of the evaporating power of the air, and it is relatively simple to estimate the rate of water loss from a surface.

Light.—The rate of transpiration is markedly increased in the presence of light. In 1877 it was shown by Wiesner that not only did direct sunlight increase the rate of transpiration, but diffused daylight and even the light of a gas lamp of 6·5 candle power also increased the rate of water loss from leaves. The gravimetric method of measuring the rate of transpiration which has already been described above was used, and a variety of different plants investigated. The results, expressed as mg. per sq. cm. of leaf surface per hour, are given in the table on the next page.

The greater amount of transpiration of the green maize plant compared with the etiolated one, led Van Tieghem some years later (1886) to coin the expression "chloro-vapourization," for

he considered this difference in rate of transpiration between green and etiolated plant to be brought about by the conversion of water into vapour under the influence of radiant energy absorbed by the chloroplasts of the leaf. Further, Wiesner claimed that green plants exposed to the influence of light of different wave-lengths showed the greatest increase in transpiration in the wave-lengths which were most absorbed. He therefore came to the conclusion that the effect of light on transpiration is due to its absorption by the tissues of the leaf, and that radiant energy is converted into heat. As a result, there will be a heating of the leaf as a whole, and as a consequence the saturation deficit in the intercellular spaces will be increased and bring about a

Material	*In Darkness Mg. per sq. cm.*	*In Diffuse Daylight Mg. per sq. cm.*	*In Sunlight Mg. per sq. cm.*
Zea Mays (etiolated) ..	106	112	290
Zea Mays (green)	97	114	785
Flower of *Spartium junceum* ..	64	69	174
Flower of *Malva arborea* ..	23	28	70

rise in the rate of evaporation. Evidence in support of this view was brought forward in 1880 by Combes, who measured the amount of transpiration in different coloured flowers and found that the highest rate of transpiration occurred under those rays that were most absorbed. It was not until 1898 that the true reason for the increase in transpiration in sunlight was shown by Francis Darwin to be due to the opening of the stomata in light. Nevertheless, light does have a small direct effect on the rate of transpiration (see below).

When the effect of light on the transpiration rate is being determined, the other factors involved must be kept constant. Under field conditions in the open it is difficult to keep such factors as humidity of the air, wind, etc., constant, and in such circumstances it is more convenient to make a simultaneous record of the rate of evaporation from some standard water surface. The rate of evaporation from a standard water surface summarizes the effect of the various external factors, and it then becomes possible to detect any differences caused by these factors on the rate of transpiration and the rate of evaporation.

Livingston* has termed the ratio of transpiration rate (T) to evaporating power of the atmosphere (E), i.e. T/E, referred in both cases to unit area, *relative transpiration*. By use of this ratio, the direct evaporating power of the air is eliminated and the physiological behaviour of the leaf is shown. Livingston determined E by use of the so-called "porous-cup atmometer." A variety of different types of these atmometers has been devised.

It was assumed by Livingston that by use of the ratio T/E, the direct influence of atmospheric conditions on the rate of transpiration may be disregarded. It was supposed that any changes in the atmospheric factors would react equally upon the transpiration rate of the plant and the evaporation rate from a water surface. The problem here is, Does the atmometer respond to these various factors in the same way as the leaf, and how far is Livingston's assumption valid? In the first place, Livingston himself, and also L. J. Briggs and Shantz,† have shown that atmometers of different sizes and shapes do not behave in the same way and are not comparable under changing conditions. It does not follow that a certain change in the environment which causes the rate of evaporation to increase twofold in one particular atmometer will bring about the same increase in another of different size and shape. Similarly the shape and size of the leaf may modify the effect of differences in external factors on its rate of transpiration. As it is not possible to compare one atmometer with another, or one leaf with another, under varying external conditions, it is scarcely valid to compare an atmometer with a plant.

R. C. Knight‡ has brought forward a further criticism in this connection based upon the internal structure of the leaf. In the leaf, a part of the route over which the diffusion stream must travel from the evaporating cells of the mesophyll tissues is within the leaf and protected by the epidermis. This being the case, it is not influenced by movement of the outside air. In the case of the atmometer, on the other hand, the whole of the diffusion stream is subject to the influence of air movements, and theoretically it is possible, if a sufficiently rapid current of air be used, to lower the moisture in the immediate neighbourhood of the evaporating surface to the same concentration as in the surrounding atmosphere. This minimal concentration can only

* *Carnegie Inst. Wash. Pub.*, 1906, **50**; *Plant World*, 1907, **10**, 269.
† *J. Agric. Res.*, 1917, **9**, 277.
‡ *Ann. Bot.*, 1917, **31**, 221, 351.

be attained on the surface of the epidermis, for the leaf and the mesophyll tissues will not be affected. In such circumstances, changes in wind velocity will have a greater effect on the rate of evaporation from an atmometer than on the rate of transpiration from a leaf. From a number of experimental determinations, Knight has shown that changes in wind velocity do affect transpiration from a leaf and evaporation from an atmometer in different ways, and it is only when wind velocity is kept constant that this method of comparison by the utilization of relative transpiration can be validly employed to eliminate changes in relative humidity and temperature on the transpira-

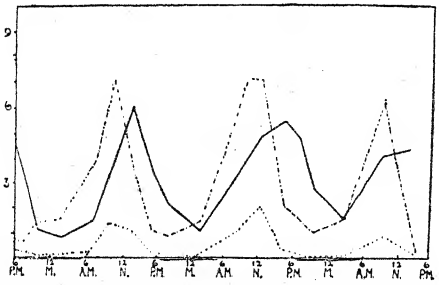

FIG. 11.—Comparison of the transpiration (broken line) of *Euphorbia capitellata* over three days with evaporation (continuous line) and the "relative transpiration" (dot-dash line). Note that the scale of the ordinates is different in the three graphs. (After Livingston, modified. From Skene, *Biology of Flowering Plants*.)

tion rate of the leaf. Provided wind velocity be maintained at a constant rate, relative transpiration will give a satisfactory measure of the intrinsic transpiring power of the plant.

Fig. 11 shows the transpiration of a desert succulent, *Euphorbia capitellata*, over a period of 3 days together with the curve for the evaporating power of the air and for relative transpiration. It will be seen that the highest rate of transpiration was reached at between 10 a.m. and 12 noon, and that this increase was followed by a steady fall. The highest point in the curve for the evaporating power of the air was obtained between 2 and 4 p.m. It is evident that the prevailing external conditions were such as to bring about maximum evaporation at this time, whereas some reaction in the plant led to a fall in water loss some hours earlier. The curve for relative transpiration reflects this result, for if both rate of evaporation and rate of transpiration had been equally affected relative transpiration should have given a

straight line graph. It is clear that rate of transpiration increased more rapidly than the rate of evaporation from the atmometer, until a maximum was attained, and the later increase in the evaporating power of the air was not reflected in the transpiration rate. A further fact that should be observed from these curves is that transpiration increases greatly in light and decreases in the dark. The presence of light brings about opening of the stomata from which the main mass of transpiration is taking place, and in the dark the stomata close and transpiration is cut down.

STOMATAL REGULATION AND TRANSPIRATION

The question of whether the rate of transpiration from a leaf is controlled by stomatal aperture has led to a good deal of controversy in the past, and the matter has only been settled within recent years. The older workers considered that stomatal aperture had an absolute control over the loss of water from a leaf, but the results of recent investigations, carried out under controlled conditions, have shown that when widely open, stomata play but a sorry part in the regulation of this process.

The precise method of measuring stomatal aperture has an important bearing on a problem of this nature. The older methods were extremely crude; the results obtained by their use cannot be regarded as reliable, and they are now merely of historical interest. One of the best known of these older methods was the "horn-hygroscope" of Francis Darwin. The horn-hygroscope consisted of a thin shaving of horn with a bristle at the tip fixed in a small piece of cork and with a scale attached. In the presence of moisture, the shaving of horn curled and the amount of curling was measured on the scale. The cobalt paper method described above can also be used. Neither the horn-hygroscope nor the cobalt chloride method measure stomatal aperture as such, they give more a measure of the rate of transpiration. It is therefore not surprising that the older investigators who used these methods came to the conclusion that there was a direct correlation between transpiration rate and stomatal aperture. Francis Darwin himself was one of the firmest upholders of this view.

Lloyd* has devised a method of measuring stomatal opening by rapidly stripping away portions of the epidermis of a leaf and

* *Carnegie Inst. Wash. Pub.*, 1908, 82.

placing them in absolute alcohol tinged with Congo-red. Lloyd claimed for his method that the walls of the guard-cells were so rapidly dehydrated and hardened by the alcohol that the stomata were not distorted in shape. Another method of measuring stomatal aperture is by means of the so-called "porometer." Darwin and Pertz* were the first to employ the porometer for this work. In this method, a current of air is drawn through the

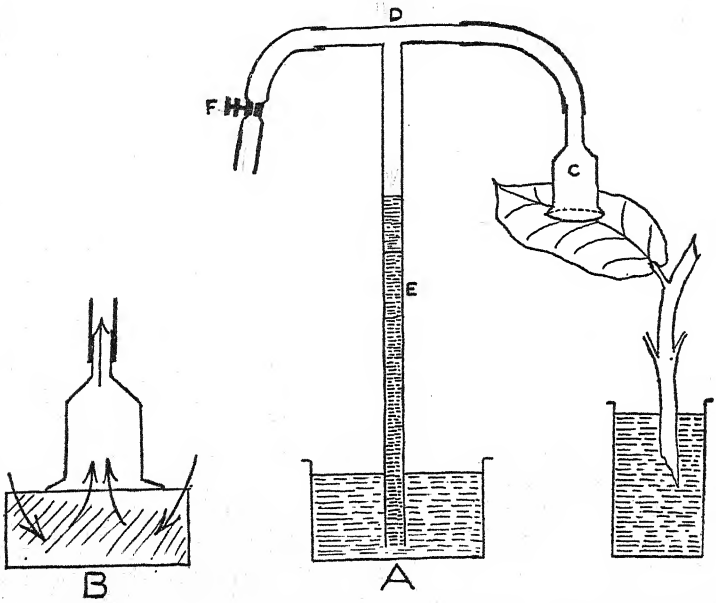

FIG. 12.—A. Darwin and Pertz porometer. For description see text. B. Path of flow of gas through leaf.

stomatal pores under a slightly reduced pressure. The actual measurement obtained is of the velocity of air drawn through the stomata of the leaf.

In essence, the Darwin and Pertz porometer consists of a small funnel C (Fig. 12A) fixed to the leaf surface by means of gelatin or other suitable adhesive. To this funnel is attached a piece of rubber tubing which in turn is attached to one arm of a T-piece, D. The lower end of the T-piece dips under mercury or water contained in a beaker, while a small-bored piece of rubber tubing with a clip F is fixed to the other arm. By opening the clip F, water or mercury can be sucked up the column E to any desired

* *Proc. Roy. Soc.* (*Lond.*), 1912, 84B, 136.

height, and the clip is then closed. A slight negative pressure is now created in the porometer chamber C, and as a result air is forcibly drawn through the intercellular spaces of the leaf via the other stomata, and the column in E will fall. The time taken by the column to fall a given distance is noted with a stop-watch. The actual path of the air current is shown in Fig. 12B. The air enters the intercellular spaces from outside the leaf via the other stomata and travels through the intercellular spaces to the particular area of the leaf surface covered by the porometer cup. It was shown by R. C. Knight that if a portion of the leaf were covered with vaseline, the resistance to the flow of air is considerably increased, for the air has to travel through a greater length of intercellular space to reach the porometer cup.

In actual practice, the Darwin and Pertz porometer has a number of disadvantages. The continual drawing of a column of liquid up the tube to a definite mark must depend on the dexterity of the individual, and the experimental results are liable to vary. This personal factor has largely been removed by means of the improved porometer devised by R. C. Knight.* With this type of porometer, the time taken for the issue of a number of bubbles is counted. In essentials, this apparatus consists of a porometer cup attached to the leaf with gelatin. The cup in turn is connected to an aspirator by means of glass tubing, and a constant pressure difference is maintained by keeping the opening of the intake tube below the surface of the water contained in the aspirator. An outflow tube leads from the aspirator, and by suitable adjustment of this outflow tube, almost any required pressure may be obtained. The end of the outflow tube dips beneath the surface of water contained in a small glass vessel. Knight found it convenient to insert a three-way stop-cock between the leaf chamber and the aspirator bottle in order to place the porometer cup in contact with the external air, and thus to release the pressure upon the leaf when no readings were being made. The time elapsing between the discharge of two consecutive bubbles from the inlet tube is taken as giving an indication of the relative aperture of the stomatal pores.

In expressing the results of porometer measurements, it is the area of the stomatal pores which must be taken into account and not their diameter. Here we are dealing with mass movements of air and not with diffusion in the Brown and Escombe sense.

* *New Phyt.*, 1915, **14**, 212.

It is therefore necessary when using the porometer for stomatal measurements to use the square root of the reciprocal of the time of bubble flow for the Knight porometer, and the square root of the reciprocal of the time for the fall of the water or mercury column in the Darwin and Pertz apparatus $\left(\sqrt{\frac{1}{T}}\right)$.

The advantages of the porometer method of measuring stomatal aperture are numerous. Firstly, a mean result is obtained, for the results of a large number of stomata are automatically integrated, and secondly, the leaf is uninjured in the process. The chief disadvantage of the porometer lies in the fact that it cannot be used with leaves possessing delicate laminae. With such leaves a slight curvature inwards occurs over the leaf chamber, and according to Knight this may affect stomatal aperture. Another disadvantage of the porometer method lies in the fact that if the current of air be passed through the pores for any prolonged length of time, the protoplasm of the guard-cells becomes irritated and as a result the stomata close.

Francis Darwin found that in porometer measurements one leaf cannot be compared with another. This fact is shown by the figures he obtained for *Prunus Laurocerasus*. The leaves from a single plant were numbered as given below.

Leaf ..	2	3	4	5	6	7	8	12	13
Time ..	6·9	15·0	14·9	5·6	14·4	6·3	19·5	140·0	78·0

The leaves 12 and 13 were formed in the previous year and were rather unhealthy in appearance.

According to Ashby,* who has carried out a comparison of Lloyd's stripping method and the porometer method of measuring stomatal aperture, the two methods do not give results which differ significantly, except at very small stomatal apertures, when the Lloyd method gives a more accurate picture of the diffusive capacity of the stomata, but with wide stomatal aperture he was able to find satisfactory agreement between the two methods.

After a long series of elaborate determinations, Lloyd† was of the opinion that there was no correlation between rate of transpira-

* *Plant Physiol.*, 1931, **6**, 715.
† *Carnegie Inst. Wash. Publ.*, 1908, **82**.

tion and stomatal opening (Fig. 13). A similar conclusion was reached by Trelease and Livingston,* who worked on *Zebrina pendula* using Livingston's improved cobalt chloride method for measuring the rate of transpiration and the porometer for determining stomatal aperture. Francis Darwin,† on the other hand, arrived at the conclusion that there was such a direct correlation between stomatal opening and rate of transpiration, and that

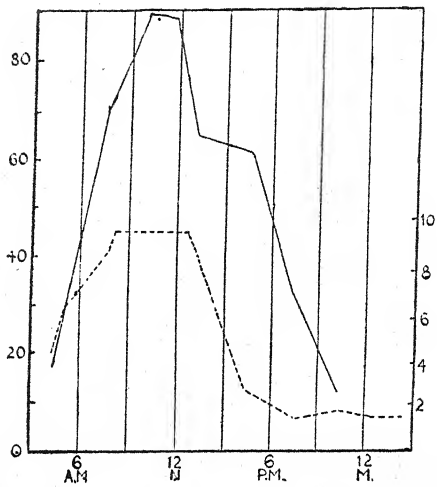

FIG. 13.—Transpiration and stomatal movement in *Verbena ciliata* compared. The continuous line represents the transpiration and the broken line stomatal movement measured in microns. (After Lloyd, modified. From Skene, *Biology of Flowering Plants.*)

stomatal aperture exercised a direct control over transpiration rate.

If the stomatal apertures are more widely open, there should be a greater loss of water from the leaf, and if the process be one of diffusion, the gradient should become steeper and altered. Within the stomata there must be an increase in vapour pressure because evaporation of water is continually proceeding from the wet walls of the mesophyll cells. It was shown by Livingston and W. H. Brown‡ that the water-content of leaves varies during the day, and the values approximately correspond to a maximum in the early morning and a minimum in the afternoon:

* *J. Ecol.*, 1916, **4**, 1.
† *Phil. Trans. Roy. Soc.* (*Lond.*), 1916, **207B**, 413.
‡ *Bot. Gaz.*, 1912, **53**, 309.

		Per cent Water
Amaranthus ..	Between 10 a.m. and 12 noon	86
	Between 5 p.m. and 6 p.m.	79
Sida	Between 7 a.m. and 8 a.m.	83
	Between 3 p.m. and 4 p.m.	75
Nicotiana ..	Between 4 a.m. and 5 a.m.	85
	Between 4 p.m. and 5 p.m.	80

The investigations of R. C. Knight* on the problem of the relationship between stomatal aperture and rate of transpiration have

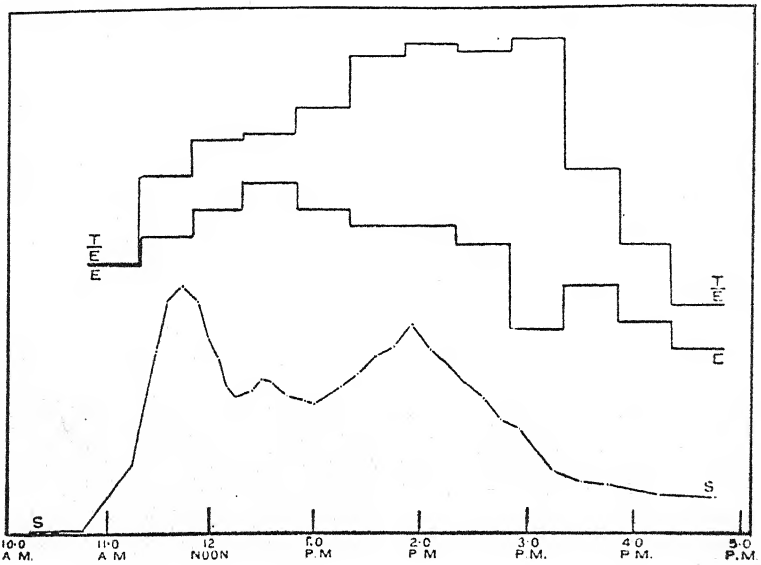

FIG. 14.—Curves showing lack of agreement between relative transpiration $\left(\frac{T}{E}\right)$ and stomatal aperture (S). E is the curve for loss of water loss from an atmometer. (After R. C. Knight.)

shed a great deal of light on this question, and are of the greatest importance in this connection.

Knight worked under absolutely controlled conditions. He found that on occasion there was complete correlation between rate of transpiration and stomatal aperture, whilst at other times no such correlation could be obtained (Fig. 14). The problem of whether or no stomata exerted control over the rate of transpiration was then attacked from a new angle. The plant was placed in a special "air-flue" (cut shoots were usually employed for this work) in which the evaporating power of the

* *Ann. Bot.*, 1916, **30**, 57; 1917, **31**, 221, 351; 1922, **36**, 361.

air could be kept at a constant value by means of an electric fan. The light intensity and temperature were also kept constant during this experimental work. Under these conditions, stomatal aperture also remained constant. The transpiration rate and evaporating power of the air also reached a constant value (Fig. 15). The fan was now stopped, when the graphs for the evaporating power of the air and rate of transpiration both

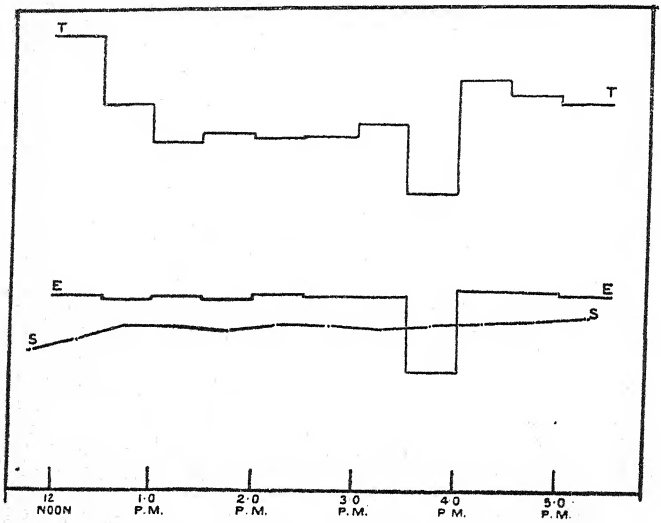

FIG. 15.—Curves showing that the rate of transpiration is increased by an increase in the water content of the leaf when the evaporating power of the air is temporarily decreased. T = transpiration; S = stomatal aperture; E = atmometer loss. (After R. C. Knight.)

showed a simultaneous fall, since layers of aqueous vapour were no longer removed from the surface of the leaves. On the other hand, the graph for stomatal aperture still remained constant and showed no fall. When the rate of evaporation and rate of transpiration had once more reached a steady value, the fan was restarted. It was found that whilst the curve for the evaporating power of the air rose to the same value as that before the fan was stopped, the curve for the transpiration rate rose to a higher value than that previously recorded. It is clear that although the rate of transpiration was reduced when the fan was stopped, water was still being absorbed by the cells of the mesophyll tissues at the same rate as before, and that therefore there was

an accumulation of water in these tissues. Thus, when the fan was restarted, the increase caused thereby in the evaporating power of the air brought about an increase in the rate of transpiration. In the atmometer, on the other hand, accumulation of water could not take place from the very nature of its structure, so that when the fan was restarted, the water loss merely rose to its former value. It is evident from the results obtained by Knight from this experiment, that it is the water-content of the mesophyll tissues of the leaf that is the controlling factor of transpiration and not stomatal aperture.

Knight further found that the increase in stomatal aperture which often occurs at the beginning of wilting is always followed by an increase in the amount of transpiration. There is, however, a fall in the rate of transpiration before the stomata have attained their maximum opening. This fall in the rate of transpiration is brought about by a fall in the water-content of the mesophyll tissues of the wilting leaf.

An extremely elaborate investigation of the behaviour of stomata under different external conditions has been made by Loftfield,* who was able to show that, at certain specific apertures stomata do exert a direct control over the rate of transpiration. Stomatal aperture was measured by Lloyd's stripping method.

Loftfield, like Knight, found that occasionally there was a direct relationship between stomatal aperture and the rate of transpiration, and at other times no such relationship could be discovered. He was able to show, "when the stomata are widely open or nearly widely open, transpiration is the result of the action of the factors of evaporation alone, since the stomata in nowise interfere with the action. As the stomata close, the influence of the factors is lessened, but until closure has reduced the apertures to 50 per cent or less, stomatal regulation is largely overshadowed by the control exerted by them. When closure is almost complete, the regulation of water-loss by the stomata is very close, and the factors overshadowed by the effect of even very small changes of the opening." This seems a very fair statement of the situation. When the stomata are widely open, it is the water-content of the mesophyll that is the important controlling factor, when the stomatal apertures are closed to 50 per cent or less, they exert a direct controlling influence on the loss of water from the leaf.

* *Carnegie Inst. Wash. Publ.*, 1921, **314**.

Francis Darwin* attempted to determine if light had any direct effect on the rate of transpiration from the mesophyll tissues of the leaf. The method employed was to smear the leaf with vaseline or some similar substance, and then place the intercellular spaces in contact with the external air by slitting the leaf between the main veins with a scalpel. Darwin concluded that the effect of light was to increase the rate of transpiration from the mesophyll by 10 to 100 per cent of the value in the dark. Either of these rates is surprisingly high, the latter especially so. Henderson† was unable to confirm Darwin's high values, but he did find that light had a small effect upon transpiration from the mesophyll, the order of increase being from 4 to 5 per cent of that in the dark. The precise action of light on the mesophyll is not known. One suggestion that has been put forward is that light may perhaps increase the permeability of the protoplasm, and decrease the resistance of the mesophyll cells to the passage of water. That light exerts a physiological as well as a physical effect on transpiration is shown by some experiments of Sablon, who found that in the variegated leaves of Pelargonium, light increased the rate of transpiration from the green leaves by 300 per cent, whereas the transpiration rate of the non-green leaves was only increased by 150 per cent.

That some secretory activity as well as simple evaporation is involved in transpiration is shown in an experiment by Dixon. A cut shoot was fixed into the narrow opening of an inverted bell-jar, so that the upper part of the shoot and the leaves projected into the interior, while the base of the shoot extended below the cork and rested in a solution of eosin. The inverted portion of the bell-jar was then completely filled with water, so that shoot and leaves were entirely submerged. In spite of the fact that water was in direct contact with the leaves, the solution of dye was quickly drawn up the stem of the shoot and extended through the veins of the leaves. It was found by Dixon that if this apparatus were placed in the dark, the eosin only rose for a short distance up the stem or sometimes not at all. Dixon considered that light exerted an indirect influence, and that it set free oxygen in photosynthesis, and the small rise of the dye shown in the dark was due to respiration and the oxygen dissolved in the water.

* *Proc. Roy. Soc. (Lond.)*, 1914, **87B**, 281.

† *Ann. Bot.*, 1926, **40**, 507.

ACTION OF ANAESTHETICS AND SALTS ON TRANSPIRATION

Dixon working with *Cytisus Laburnum* and *Syringa vulgaris* found that many gases and anaesthetics have a marked effect on the rate of transpiration. If the specific amount transpired in air was given the value 100, it was discovered that the following values were obtained in the presence of the substances tabulated in the list given below:

	Per cent
Oxygen	105
Air	100
Carbon dioxide	87
Ether	82
Chloroform	66

It has also been found that the addition of various salts, either to the soil or to culture solutions, markedly affects the rate of transpiration. It was first shown by Sachs, and later confirmed by Burgerstein, that the addition of tartaric, oxalic and nitric acid to the soil decreases the rate of transpiration, whereas the addition of ammonium, sodium and potassium salts increases the rate.

STOMATA AND THE MECHANISM OF STOMATAL MOVEMENT

It has already been seen that the stomata are the chief path of gaseous exchange for the leaf, and the part that they play in transpiration has been discussed. The mechanism of stomatal movement will now be considered.

The stomata of different plants show very little variation in their general structure. They consist of two sausage-shaped cells, the guard-cells and a central pore. The stoma is a very ancient type of structure, and is found to be of practically the same shape in plants living as far back as the Devonian as in plants of to-day. The stomata of the Rhynie fossils, for example, are but little different from the stomata of modern plants.

Stomata occur most abundantly on leaves. In mesophytes, they are almost entirely confined to the lower surface of the leaf, but in floating plants, e.g. Nymphaea, they occur on the upper surface. In completely submerged plants, they are usually entirely absent or only show as rudimentary structures.

It was first observed by von Mohl that the opening and closing of stomata was dependent on changes in turgidity of the guard-cells. The outer walls of the guard-cells are thin, whereas the inner layer abutting on the pore is thickened. Any increase

in the turgor of the guard-cells causes a considerable distension of the outer convex surface, whilst the inner concave surface is not so extensible. This increase in the turgidity of the guard-cells and expansion of the convex surface causes the pore of the stoma to open, and with loss of turgor closure takes place.

In dicotyledons, it is the general rule that the outer convex wall of the guard-cell separating it from the neighbouring epidermal cell is thin-walled and the inner wall is thickened, e.g. Helleborus, whereas in the Gramineae and Cyperaceae the structure of the stomata is somewhat different, the opposite ends are thin-walled and the ridges in contact thickened. With increase in the turgidity of the guard-cells, the thin-walled end portions expand, and the middle rigid region which remains straight, is pulled apart.

The most important factor which brings about stomatal opening is light. It was discovered by von Mohl and Schwendener that as a general rule stomata open in the light and close in the dark. On the other hand, Leitgeb found that in certain plants the stomata remain more widely open at night. The stomatal behaviour of different plants towards light varies considerably, and Loftfield* has recognized three classes:

(1) *Cereals.*—The stomata in this class are sensitive to conditions of evaporation, temperature and water-content, and during daylight the stomatal aperture is dependent in duration and degree upon these factors. During the night the stomata remain closed under normal conditions.

(2) *Thin-leaved Mesophytes.*—The general rule in this class is that the stomata remain open during the day and close at night. Under extreme conditions the stomata may remain closed all day and open all night, the actual degree of opening depending on the water-content. In Alfalfa, for example, under normal conditions, the stomatal behaviour is as follows: The stomata open from two to six hours after daylight, remain open for a period varying between three and six hours, and then gradually close during a period which is approximately twice as long as that required for opening. If conditions during daylight hours should for any reason become unfavourable, partial or even complete closure may occur during the middle of the day, and if external conditions should become very unfavourable the stomata may not open during the whole of the day.

* *Carnegie Inst. Wash. Publ.*, 1921, 314.

(3) *Fleshy-leaved Plants.*—Loftfield also includes in this group some thin-leaved plants, but the potato may be taken as typical of this third group. Here the stomata are widely open during the day and night. With increase in the evaporating power of the air beyond a certain point, the stomata tend to shut. Loftfield has recognized three sub-groups in this class, depending upon time of closure. In the potato, closure of the stomata takes place for a time immediately after sunset, but as the water-content decreases, the time of closure may be extended back into the afternoon. In the cow-beet, under favourable conditions, closure may take place for a short time during the night, but the stomata remain widely open during the day. In the third sub-group, of which the onion may be taken as an example, under conditions of high water-content and low evaporation, the stomata remain widely open at night; if the water-content becomes low, the stomata close at night, but should the evaporation increase instead, the stomata tend to close during the day.

It has already been seen that the early work of von Mohl and Schwendener showed that stomata are markedly sensitive to the action of light, and that the opening of the pores is dependent upon changes in turgor of the guard-cells. Von Mohl noted the presence of chloroplasts in the guard-cells, and thought that the turgor of the latter was increased by the photosynthetic activity of these plastids in the light, with the formation of soluble sugars and increase in the osmotic pressure of these cells. Leitgeb found, however, that the stomata still opened in light in the absence of carbon dioxide. Moreover it was shown by Lloyd, Iljin and Loftfield that the opening of the stomata in light is followed by a decrease in the starch-content of the guard-cells, and when the stomata closed, the starch-content increased (Fig. 16). The guard-cells behave in exactly the opposite way to the mesophyll cells of the leaf in the presence of light. In dicotyledons, the assimilating cells of the mesophyll layer of the leaf synthesize sugars in the presence of light, and these sugars are temporarily stored as starch; with continuance of photosynthesis there is an increase in the starch-content of the leaf. Fig. 16 shows the relationship between starch-content of the guard-cells and stomatal opening for the Lombardy poplar.

The decrease in the starch-content of the guard-cells and its hydrolysis to soluble sugars will lead to an increase in the osmotic pressure of the guard-cells, and the necessary condition for opening

of the stomata will be present. A comparison has been made by Wiggans* of the osmotic pressure of the guard-cells and epidermal cells for different plants. In general terms, the osmotic pressure of the epidermal cells remained constant during the period in which they were examined, whereas the osmotic pressure of the guard-cells showed marked fluctuations. In Cyclamen, for

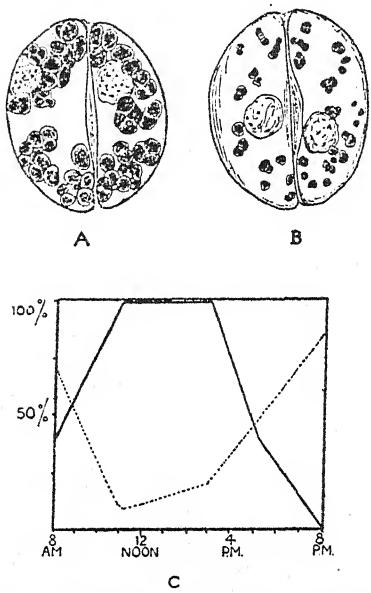

FIG. 16.—A. Guard-cells of *Fouquieria splendens* showing abundant starch grains (closed condition at night). B. The same guard-cells showing decrease of starch grains. (Open condition in the light.) C. Starch-content and stomatal movement in the leaf of Lombardy poplar. The broken line shows the changes in starch-content, the continuous line stomatal movement. (A and B after Lloyd, C after Loftfield, modified. From Skene, *Biology of Flowering Plants*.)

example, the guard-cells showed an osmotic pressure of 14·6 atmospheres at 7 a.m., which rose to a maximum of 31·0 atmospheres at 11 a.m., and the value then slowly fell to 18·5 atmospheres at 5 p.m. Throughout this period the osmotic pressure of the epidermal cells remained at 10·2 atmospheres. In the same way, in the beet, the osmotic pressure of the guard-cells rose from 23·5 atmospheres at 7 a.m. to 31·6 atmospheres at 11 a.m., the pressure remained constant at this value until 1 p.m. and then fell away to 25·0 atmospheres at 5 p.m. Again, throughout this

* *Amer. J. Bot.*, 1921, 8, 30.

period the epidermal cells showed a constant value of 12·5 atmospheres. A similar condition was found in *Rumex Patientia* by Sayre.* When the stomata were shut at night, the guard-cells showed an osmotic pressure of 13 to 14 atmospheres, but when the stomata were open, the value of the osmotic pressure rose to 23 atmospheres. The osmotic pressure of the subsidiary cells remained constant at 15 atmospheres, whilst the epidermal cells showed a lower but constant value of 13 atmospheres.

It has been supposed that the conversion of starch into sugar and sugar into starch in the guard-cells, is in the nature of a reversible enzymic reaction, and that this reaction is in some at present unknown way conditioned by light. Lloyd and also Loftfield state that under screens of blue glass, stomata open, and the hydrolysis of starch into soluble sugars proceeds normally, whereas blue light lowers the rate of photosynthesis. It has been claimed by Baly and Semmens† that polarized light markedly increases the hydrolytic activity of diastase on starch, and it may be that since normal daylight contains a considerable amount of polarized light, this may have some influence on stomatal opening. Neilson Jones,‡ however, has denied that polarized light has any such stimulating influence on diastase, and though it must be confessed that the technique employed by Baly and Semmens was open to the very justifiable criticisms that were made against it by Neilson Jones, nevertheless, it would be of interest to have this work repeated under properly controlled conditions, for if it be true it explains a good deal that is at present obscure.

F. Darwin, Leitgeb and Lloyd claimed that a reduction in the partial pressure of the carbon dioxide surrounding a leaf led to the opening of the stomata, and Linsbauer§ has shown that an increase in the partial pressure of this gas brings about closure. According to Weber,|| the regulation of the concentration of the carbon dioxide in the guard-cells is brought about by its photosynthetic absorption.

According to Sayre¶ and Scarth,** stomata open in acid and also alkaline medium, but the opening is greater in the acid medium. In *Zebrina pendula*, for example, it was found by Scarth that in an intermediate range of pH, i.e. from 5·5 to 7·0, the stomata remained shut, but whether there was an increase in the

* *Ohio J. Sc.*, 1926, **26**, 233.
† *Proc. Roy. Soc.* (*Lond.*), 1924, **97B**, 250.
‡ *Ann. Bot.*, 1925, **39**, 651.
§ *Flora*, 1917, **109**, 100; *Planta*, 1926, **2**, 530.
|| *Naturwiss.*, 1923, **17**, 309; *Ber. deut. bot. Ges.*, 1927, **45**, 408.
¶ *Science*, 1923, **56**, 205.
** *Plant Physiol.*, 1926, **1**, 215.

acid or alkali direction, the stomata opened until the limits of injury were reached. It was also found that in the pH zone in which the stomata remained closed, starch made its appearance in the guard-cells, but disappeared in that of opening, and that these changes were reversible. Further, it was shown by Scarth that when the stomata were open the pH of the guard-cells was higher than when they were closed. This range of pH was from 7·0 to 4·5.

The plastids of the guard-cells are apparently more of the nature of leucoplasts than true chloroplasts. Thus, Kümmler* showed that the guard-cells of the stomata of white-margined Pelargonium contain abundant starch, more in fact than the guard-cells of normal green leaves, and these stomata open in the light and close in the dark. It is evident that chlorophyll is not an essential part of these plastids.

Temperature has an important effect on the rate of movement of stomata. Loftfield† showed for Alfalfa that the times of opening of the stomata in light for the following temperatures, 1° C., 10° C., 20° C. and 30° C. was 6 hours, 4 hours, 2 hours and 1 hour respectively. Thus, a rise of 10° C. in the temperature has doubled the rate of movement. This is to be expected if the reaction of conversion of starch into soluble sugar is enzymic in nature.

Stomatal Behaviour at Wilting.—A number of conflicting statements have been made about the behaviour of stomata at wilting of the leaf. F. Darwin‡ stated that the stomata at the first inception of wilting open widely, and this is followed by closure. R. C. Knight,§ who worked under controlled conditions, has been able to confirm this statement. It is only when wilting is very pronounced that closure takes place (Fig. 17).

Stomatal Rhythm.—F. Darwin|| arrived at the conclusion that there was some internal rhythm that controlled stomatal movement. He followed the stomatal movement of *Prunus Laurocerasus* throughout 24 hours and discovered that the stomata began to open about midnight. Knight was able partially to confirm this result, but it has received full confirmation at the hands of Maskell|| who used *Prunus Laurocerasus var. rotundifolia* with the

* *Jahrb. f. wiss. Bot.*, 1922, **61**, 610.
† *Carnegie Inst. Wash. Publ.*, 1921, 314.
‡ *Phil. Trans. Roy. Soc.* (*Lond.*), 1898, **190B**, 531.
§ *Ann. Bot.*, 1917, **31**, 221, 351.
|| *Proc. Roy. Soc.* (*Lond.*), 1928, **102B**, 467, 488.

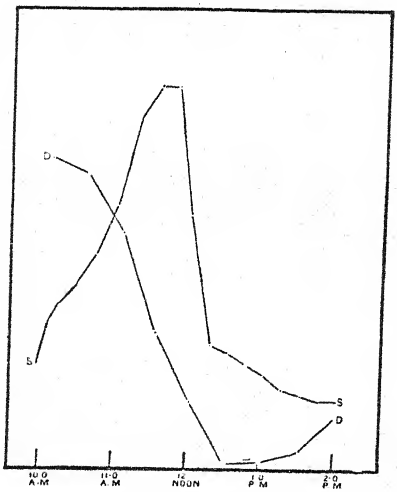

FIG. 17.—The behaviour of stomata at wilting. The stomata show increasing aperture with increasing water-loss. D = water deficit; S = stomatal aperture. (After Knight.)

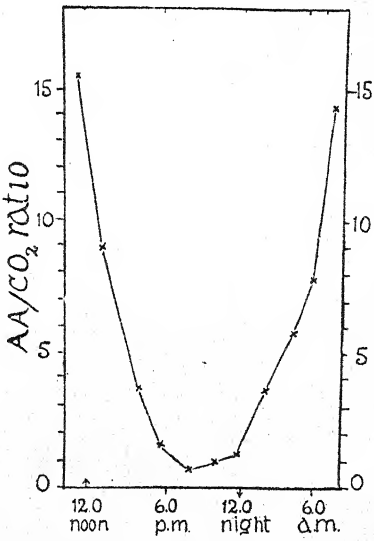

FIG. 18.—The march of apparent assimilation in leaves of Cherry Laurel under conditions of continuous artificial illumination. (After Maskell, modified. From Barton-Wright. *Recent Advances in Plant Physiology.*)

continuous current method of estimating photosynthesis and the porometer for estimating stomatal aperture. In constant intensity of light and low concentrations of carbon dioxide it was found that there was a diurnal rhythm in photosynthesis. The photosynthetic rate fell during the afternoon and evening and rose again during the morning. The apparent rather than the real assimilation (see Chapter VIII) was measured, as it was the uptake of carbon dioxide that was principally being estimated, and a good picture of the march of assimilation was obtained by calculating for each reading the ratio between the apparent assimilation and the mean carbon dioxide concentration. The results are graphically shown in Fig. 18. Simultaneous observations on the porometer and photosynthetic rate showed these results to be due to the march of stomatal aperture.

It will be seen that the opening and closing of stomata is a highly complex physiological phenomenon, and our knowledge of the exact mechanism involved is still very limited.

TRANSPIRATION AND XEROPHYTES

Xerophytes may be defined as plants adapted to live under dry conditions of air and soil. This term was first introduced in 1822 by Schouw. Had botanists been content to adhere strictly to this definition, the evident confusion that has surrounded this subject for thirty or more years would not have occurred. The main source of this confusion must be traced to Schimper, who in his book *Plant Geography*, first published in 1898, made an attempt to classify vegetation according to adaptation to habitat.

Schimper classified plants living under a deficient water supply as xerophytes, and pointed out that such plants have numerous adaptations for cutting down transpiration to a minimum, and also possess a high osmotic pressure so that they are able to extract a maximum amount of water from the soil. In plants living under desert conditions, he emphasized the peculiar structures that were to be found, e.g. succulents, plants with reduced leaf surface, hairs on the leaves, sunken stomata and plants with thick cuticles. But plants living under other than desert conditions possess many of these structural peculiarities. Plants growing in salt marshes are succulent, heath plants are microphyllous, and mangrove plants have leathery leaves and sunken stomata. There are two possibilities, either to consider that these characters are not

significant to xerophytism, or to include marsh plants, halophytes and heath plants in the group of xerophytes. Unfortunately, Schimper chose the latter course, and coined the phrase beloved for so long by ecologists, *physiological drought*, which did duty for a number of years in place of experimental proof. Although water was present in these habitats, it was not supposed to be readily available to plants on account of the high osmotic pressure of salt solutions, bad aeration in marshes, and toxins in moor and heath habitats.

Succulents.—Succulents of the Cactus type are the only plants for which the original conception of xerophytism holds. Such plants possess a massive parenchymatous tissue well adapted to store large amounts of water. Their osmotic pressure is low, rarely more than 10 atmospheres, and they differ in this respect from halophytes (see below), which have a high osmotic pressure. They also possess a superficial root system which enables them to absorb water even when the supply is scanty, although a few have deep roots. They possess a thick cuticular covering and relatively few stomata. The stomatal behaviour is peculiar in these plants, for the stomata close during the day and open at night, and their relative transpiration is lower by day than by night.

The fact that the stomata of these desert succulents close during the day and open at night to conserve water supply, leads to complications in their metabolism. If the stomata close during the hours of light, the supply of carbon dioxide to the assimilating cells will also be reduced at the time when assimilation can take place. The incomplete respiration of these plants, and the manner in which carbon dioxide is liberated for photosynthesis, will be considered in Chapter XIII.

The Pentosan Theory.—It was first suggested by McDougal that the protoplasm of succulents consisted "in the main" of pentosans, since pentosans and protoplasm exhibit or were considered to exhibit a similarity in their hydration properties. Examination of succulents showed that they contain a certain amount of pentosan, and it was concluded that high temperatures and arid conditions were favourable for the formation of these substances. Succulency was considered to be due directly to the conversion of hexose polysaccharides into pentosans and mucilage, for the latter have a high coefficient of imbibition, while in the former the value of the coefficient is low.

The main objection to this theory is that the pentosan-content of succulents is very variable, and many non-succulents also contain pentosan. It is difficult to believe that a slightly greater amount of pentosan in succulents as compared with non-succulents is sufficient in itself to explain the widely differing features of this group. Pearsall and Ewing* have correlated succulence in plants with high nitrogen supply. They found that plants grown under rich conditions of nitrogen manuring possess a low protein and high amino-acid content, and the pH of the tissues is also higher. This high pH and high amino-acid content is considered to lead to a greater swelling of the protoplasmic colloids, and it is perhaps due to this feature that there is a higher water-content and reduced transpiration of plants growing under conditions of high nitrogen manuring. Chapman, on the other hand, working with *Tradescantia fluminensis*, considers that succulence is produced by withholding nitrogen and iron, and the presence of excess of potassium salts.

The Cacti store water in such quantities that they are never short of it, and with their strong reserve they are not faced with the problem of drought. Burgerstein has shown that the ratio of transpiration from the stem of Opuntia and the leaf of Hydrangea, a typical mesophyte, is approximately 1 to 32.

Halophytes.—These plants are succulents, and their succulence was supposed by Schimper to be due to physiological drought caused by the high osmotic pressure of the soil solution. The idea that halophytes have a reduced rate of transpiration was accepted for many years without question, largely on Schimper's authority. This is certainly odd, for Stahl, using his cobalt chloride method, had found that halophytes show a considerable and sustained rate of transpiration. He asserted that the stomata in these plants have lost the power to close, a fact that Rosenburg failed to confirm. Delf† has shown that halophytes are able to remove water from saline solutions without difficulty, and transpire rapidly, and that they wilt and die if exposed to drought. Their resemblance to real xerophytes, e.g. desert succulents, is merely superficial. Working with a variety of halophytes, such as *Salicornia annua*, *Atriplex portulacoides* and *Suaeda maritima*, Delf compared the rates of transpiration with typical mesophytes (*Mercurialis annua* and *Vicia Cracca*), and a few of her observations are given on the next page.

* *Ann. Bot.*, 1929, **43**, 27.

† Ibid., 1911, **25**, 485.

Loss of Water per Hour per 100 cm²

	Grams
Mercurialis annua	0·066
Salicornia annua (green form)	0·173
Salicornia annua (red form)	0·226
Suaeda maritima	0·350

The relative transpiration of Salicornia, Sedum and *Vicia Cracca* was also compared with that of a free water surface:

	Free Water Surface = 100
Sedum spurium	36
Salicornia annua	32
Vicia Cracca	26

Delf concluded that halophytes could not be classed as xerophytes, and that the succulence of these plants allowed them to transpire at a high rate in proportion to their surface.

Heath and Bog Plants.—The investigations of Montfort* showed that the normal transpiration of such a plant as Eriophorum is not inhibited by the addition of bog toxins to the water. In fact, bog plants do not live under conditions of physiological drought, and vegetation living in this habitat is not xerophytic. The only reason that such plants have to be included in the class of xerophytes is that many possess anatomical structures which are commonly found in desert plants. Montfort drew the distinction between *xerophytes*, i.e. plants which live under conditions of inadequate water supply, and *xeromorphs*, plants which have structural characteristics usually associated with xerophytes.

Stocker† has shown that neither moor nor salt marsh plants are xerophytes. He ascertained that plants like *Erica Tetralix* and Calluna have not got a reduced leaf area relative to the absorbing area of the roots, and that their transpiration calculated on this basis is as high as that of mesophytes. It is true that the leaves have xeromorphic characters, and although transpiration from individual leaves may be reduced, there are so many of them to each plant that they do not reduce water loss. The microphyllous character of the leaves was thought by Stocker to be an adaptation to the drying winds that sweep across German heaths, and that their xeromorphic type acts as a partial compensation for the increased leaf area.

Reduction in the size of leaves has been regarded as one of

* *Zeit. f. Bot.*, 1918, **10**, 257; *Jahrb. f. wiss. Bot.*, 1921, **60**, 184.
† Ibid., 1923, **15**, 1.

those features which are an adaptation for the reduction of transpiration. But plants with microphyllous leaves are usually extremely richly supplied with leaves, and a sufficiently large number will make up for their small size. Thoday* has discussed the problem of extreme cases of dissected leaves with narrow linear or filiform segments, as well as pinoid, ericoid and cupressoid leaves. He has pointed out that in such leaves no part of the mesophyll is more than a very short distance from the main channel of supply, and that reduction of internal resistance is of more importance than reduction of surface. The centric type of leaf, with its palisade cells radiating directly from the central bundle, may be regarded as reducing resistance to water flow within the mesophyll to a minimum.

Sclerophylls (*Sclerophytes*).—It is in this class of plants, which are perennials, and have no device for storing water, that the common xeromorphic characters are to be found. Nevertheless, these structural peculiarities do not reduce the rate of transpiration.

Sclerophylls, as a class, are characterized by a thick cuticle on the upper surface of their leaves, and an abundance of supporting mechanical tissue. They have a wide distribution in the desert regions of the Old and New World and along the coastal regions of the Mediterranean sea. The vegetation bordering on the Mediterranean is exposed to two periods of drought in the year. The first period is in the early summer months, and the second occurs in the somewhat mild winter. In this second period of drought, owing to the cooling of the soil, transpiration exceeds water absorption.

Maximov† and his co-workers have made a thorough examination of the transpiration of these sclerophylls, and compared them with typical mesophytes. He came to the remarkable conclusion, that in spite of their lack of water supply, reduced leaf area, sunken stomata and thick cuticular covering, sclerophylls as a class transpire *more* rapidly than mesophytes. It is the true succulents of the Cactus class that conserve their water supply. A few of the results obtained by Maximov, at the Russian experimental station at Tiflis, are given on the next page. The term *intensity of transpiration* employed by Maximov is defined as the amount of water lost by a plant in unit time, per unit of transpiring

* *J. Ecol.*, 1931, **19**, 297.

† In this connection Maximov's work, *The Plant in Relation to Water*, should be consulted.

surface (usually the leaves). Burgerstein has suggested that the hour be taken as the unit of time, square decimetre as the unit of transpiring surface, and the gram as the unit of weight of water transpired.

It is clear from the figures given in this table that in general sclerophylls possess a higher intensity of transpiration than mesophytes. Three of these xerophytes, *Verbascum ovalifolium*, *Stachys Kotschyi* and *Helichrysum candidissimum*, possess leaves densely

	Intensity of Transpiration	*Rapidity of Expenditure of Stored Water in the Leaf*	*Type of Leaf*
(A) *Xerophytes*—			
Sedum maximum ..	2·8	8	*Succulent*
Zygophyllum Fabago	4·9	15	*Semi-succulent*
Verbascum ovalifolium	8·8	71	*Densely hairy*
Salvia verticillata ..	9·9	55	*Hard fleshy*
Falcaria vulgaris ..	18·7	87	*Hard, covered with wax*
B. Mesophytes—			
Lamium album ..	3·6	58	*Shade plant*
Viola odorata ..	4·0	58	*Sun plant*
Erodium ciconium ..	9·2	83	*Sun plant of spring vegetation*

covered with hairs, and the leaves of *Falcaria Rivini* and *Glaucium luteum* have a thick cuticle and are covered with wax. The facts presented here show that these characteristics have little significance as a protection against excessive transpiration and are only of value at wilting.

Ashby* has made an investigation of the transpiratory organs of the creosote bush (*Larrea tridentata* or *Covillea tridentata*) from the same standpoint as Maximov. This plant is an inhabitant of the driest regions around Tucson in the Arizona desert. It is a perennial with small, sticky, resinous leaves, oppositely arranged along a much-branched stem. The transpiration of *L. tridentata* was compared with that of ordinary privet. In the first place it was found that the leaf area of *L. tridentata*, far from being reduced, has in point of fact a greater area than privet. The stomatal area was also found to be greater than that of privet. Larrea was

* *Ecology*, 1932, **13**, 182.

found to be much less economical as regards water loss than privet, and lost as much water in a day as a privet bush of comparable size, provided that the stomata remained open.

Sclerophylls are characterized anatomically by decrease in the size of the cells, strong mechanical development of palisade tissue, denser network of veins and an increase of stomata per unit area of leaf surface. They possess a high osmotic pressure and a high intensity of transpiration. Maximov has suggested that they are able to withstand prolonged shortage of water supply, not by reducing their intensity of transpiration, but by the fact that their protoplasm is able to withstand desiccation without injury. Larrea, during dry periods, has no method of storing water within its tissues and no external supply of water. Under these conditions it actually wilts. The leaves become flaccid and fall off, and the plant remains in this wilted condition until the advent of the rains. After a few hours of rainfall the plant revives and transpires vigorously. The presence of xeromorphic characters in these sclerophylls, such as strong development of mechanical tissue, prevents complete desiccation and collapse of the leaf once wilting has commenced, but they are, nevertheless, extremely extravagant with their water supply. Under similar conditions, a mesophyte like privet, wilts beyond recovery, while Larrea recovers at the first adequate shower of rain.

The question naturally arises as to why desert plants should be so improvident with their water supply. The greater number of stomata in relation to size of plant is one reason for increased transpiration, but such a character may possibly be of advantage to desert vegetation, for the transpiration and assimilation are closely correlated. The plants cannot have the one without the other. The rainy periods offer to the sclerophylls the one opportunity for the manufacture of carbohydrates, when the stomata are open and the leaf is no longer in the wilted state.

The main point at issue is that sclerophylls are adapted to their habitat by virtue of the fact that their protoplasm is able to endure prolonged periods of desiccation. The problem of how they accomplish this condition has yet to be solved.

XEROMORPHY

The question can be conveniently discussed here as to the significance of xeromorphic characters and why they should be

associated with xerophytes. In the first place, xeromorphy can be induced experimentally. Rippel,* for example, has shown that with a mesophyte, such as *Sinapis alba*, grown in soil of different water-content, xeromorphic features are induced in the drier soils. The leaves develop smaller cells, fewer intercellular spaces, more mechanical tissue and a greater number of stomata per unit area of leaf surface.

The next matter that has to be considered is, What effect do these xeromorphic features have on the rate of transpiration? Maximov and his co-workers have shown that more xeromorphic leaves transpire more rapidly than less xeromorphic leaves. Some observations of Zalenski are of great importance in this connection. As far back as 1904, he observed the fact that successive leaves on a stem, starting from the base of the plant, become more and more xeromorphic in character. He showed that there was decrease in cell size, and therefore an increase in the number of stomata per unit area; if hairs were present on the leaf there was an increase in their number; and also there was a greater development of wax and cuticle, an increased development of mechanical tissue and a decrease in the size of the intercellular spaces. Yapp,† working quite independently, obtained similar results for *Spiraea Ulmaria*. Yapp was of the opinion that the increase in xeromorphic characters in the upper leaves of a plant are, in part at any rate, due to differences in turgor in the cells of lower and upper leaves during their development. The leaves in the upper parts of the plant develop during the summer months and are in a drier region of air, and in these circumstances, conditions are suitable for greater transpiration, whereas the lower leaves develop during the spring. The suggestion has been put forward by Yapp that the water supply to the upper leaves may be partially deflected into the mature leaves, which are of course continually increasing in number. Thus, as each new leaf is formed, there will be greater resistance in the water passage between itself and the root; and at the same time there will be a greater competition for water with the leaves that have already formed, so that the turgor of the upper leaves is less than the turgor of the lower leaves.

A biochemical suggestion has been put forward by Walter to account for the effect of reduced turgor on cell size. He has pointed out that in a leaf there is a "physiological equilibrium"

* *Beih. Bot. Centralbl.*, 1919, **36**, 187. † *Ann. Bot.*, 1912, **26**, 815.

between starch and sugar (starch $\rightleftharpoons$ sugar), which depends upon the water-content. When the water supply is adequate, the direction of the reaction is from sugar to starch; but should the turgor of the cells fall, then the reverse reaction takes place, and starch is hydrolysed to sugar. As a result of the increase in soluble sugars, the osmotic pressure of the cells will be increased. In the cell there is an equilibrium between the water held osmotically in the cell sap and the water held by imbibition in the protoplasm. When the osmotic pressure of the cell sap increases, water will be removed from the protoplasm, there is a "de-imbibition" of the protoplasm. Walter has suggested that when the protoplasm is in a partially desiccated condition, normal cell size cannot be attained, hence in xerophytic leaves is found a smaller size of cell, more numerous stomata and increased transpiration.

SIGNIFICANCE OF TRANSPIRATION

All land plants transpire. The question arises as to whether the process is beneficial or harmful to the land plant. A variety of different views on this problem have been taken by different investigators, but whether or no the process be harmful or beneficial, it is quite unavoidable. Since the green plant synthesizes carbohydrates from carbon dioxide in the light, it must possess a suitable mechanism for gaseous exchange, and this function is mainly performed by the stomata of the leaf. If stomata be present, water loss must also take place.

Barnes has described transpiration as an unavoidable evil, and Curtis has concluded that it is almost entirely harmful in its effects. One favourite explanation that transpiration is of benefit to plants is that, in plants exposed to extreme conditions of heat, transpiration cuts down excess of temperature. This may be true within limits but it cannot apply to succulents like the Cacti, which possess a massive structure and thick cuticle which prevents rapid transpiration. It has also been shown by Askenasy that the temperature of the internal tissues of these plants may be as much as 20° C. above that of the surrounding air. In ordinary mesophytes, the temperature of the leaves is not more than a degree or so above that of the surrounding atmosphere, and in high winds the temperature may fall below that of the air. If the leaves be cut, the rise in temperature from "wound-shock" response is only one or two degrees. In any event, the shape of

an ordinary dorsiventral leaf, with its flat surface and large surface area exposed to the air, would prevent any undue rise in temperature should external conditions of drought or heat arise.

Miller and Saunders* and Clum† have made careful measurements of the relationship between rate of transpiration and leaf temperatures under conditions of limited water supply in a variety of different plants. In the cow-pea, it was found by Miller and Saunders that in one case the temperature of the surrounding air was 37·0° C., while in the turgid leaf it was 37·5° C. and in a wilted leaf 46·0° C., and the maximum difference between the rates of transpiration of turgid and wilted leaves was 20 : 1. In general terms, the temperature of turgid leaves was but little different from that of the surrounding air, whereas wilted leaves had a higher temperature. These results give some credence to the view that transpiration may possibly play some part in reducing leaf temperature. Clum, on the other hand, could find no such evidence.

Another claim has been made that transpiration is of benefit to the plant in that it gets rid of useless water and allows of the rapid absorption of salts from the soil. The process of transpiration has nothing to do with salt absorption. Hasselbring,‡ Mendiola,§ Muenscher|| and others have shown that there is no proportionality between salt absorption and rate of transpiration. The absorption of salts from the soil is a question of the salt and water equilibrium of the cell, and is not connected with the evaporation of water from the leaves. Once, however, salts have reached the xylem, and the xylem is their channel of upward transport in the plant, it is possible that transpiration may increase their rate of transport from one part of the plant to another.

ROOT-PRESSURE AND GUTTATION

The excretion of fluid is a common phenomenon in the plant world, and may take place either from the intact plant, or after injury, as in the "bleeding" of cut stems. This exudation of water falls into two well-marked types, *bleeding* and *guttation*.

In the case of bleeding, it is a well-known fact that if the stems of many plants be cut, especially in the spring, there is a copious

* *J. Agric. Res.*, 1923, **26**, 15.
† *Amer. J. Bot.*, 1926, **13**, 194, 217.
‡ *Bot. Gaz.*, 1914, **57**, 72, 257.
§ *Phillipine J. Sci.*, 1922, **20**, 639.
|| *Amer. J. Bot.*, 1922, **9**, 311.

flow of liquid which is excreted under pressure. This is usually spoken of as *root-pressure*, a better term would be *bleeding-pressure* or *exudation-pressure*, for although it is true that roots excite the most active exudation of water, this is because they are the best absorptive organs possessed by the plant. The root-stock does not, as a matter of fact, always bleed more actively than portions of the stem, and in a number of cases it has been found that active bleeding will take place in stems severed from the root.

Bleeding-pressure was first demonstrated in 1748 by Stephen Hales. He found that when he attached a mercury manometer to the root stump of a severed vine shoot and watered the root, a pressure of 107 cm. of mercury was developed. The amount of bleeding, and the pressures registered, vary enormously in different plants. Some values are given below:

	Centimetres of Mercury
Petunia	0·7
Chenopodium	1·6
Ricinus	33·4
Urtica dioica	46·2
Vine	90·0–110·0
Birch	140·0

The fluid that escapes in bleeding may be either practically pure water as in the potato and sunflower, or may contain considerable amounts of organic matter. Thus the sap of *Acer platanoides* contains from 1·15 to 3·4 per cent of cane sugar. Albricht examined the sap exuded from cut potato plants over a period of five days, the exudation from each day was collected separately and analysed. The figures obtained expressed as mg. per litre are given below:

Days	1	2	3	4	5
Combustible matter ..	450	310	220	280	295
Ash	1,160	980	960	910	945
Total dry weight ..	1,610	1,290	1,180	1,190	1,240

The total solids discovered in the exudate in this case were mainly mineral. Schroeder has examined the composition of the sap exuded from a birch tree, and found there is a far greater preponderance of organic matter compared with ash. He also dis-

covered that the composition of the sap was different in the spring from that of the early summer; the values obtained are given below, again expressed as mg. per litre:

	Sugar	*Protein*	*Malic Acid*	*Ash*
April 5	12,500	—	—	—
April 11	13,500	—	320	500
April 17	10,900	21	—	640
May 2	10,100	6	—	1,080
May 19	9,400	6	437	—
May 22	6,900	—	—	—

It will be seen from these figures that more organic matter is exuded in the spring. It has been suggested that during the summer there is an accumulation of organic matter in the wood and that it is rapidly removed to the growing region in the spring. After the leaves have developed the ascending sap contains mainly inorganic salts.

The sap in bleeding is exuded from the xylem; bleeding, therefore, corresponds to filtration under pressure, and the exuding liquid may or may not be mixed with bubbles of air. There is great variation shown in the duration of outflow of sap. In *Arenga saccharifera* it lasts for several years, in Agave for four or five months, while in small herbaceous plants the exudation only lasts for a few days. The amount of sap exuded increases gradually to a maximum and then falls away, and similarly the pressure also rises to a maximum and then falls. The actual amount of sap which exudes from different plants shows very large variations. Thus, *Begonia coccinea* gave 168 c.c. in 29 days, *Helianthus annuus* 30 c.c. in 16 days, while at the other end of the scale we have such plants as birch, in which approximately 30 litres have been found to be given out in the short time of eight days, and in the Agave nearly 1,000 litres of liquid are given out in the course of bleeding, usually at the rate of 3 to 6 litres a day. During the progress of bleeding it has been found that plants exude a greater volume of liquid than the total volume of their roots. Hence it follows that water must be taken in from external sources to keep up the flow. The two values given overleaf show how different is the volume of the roots from the volume of water exuded.

	Volume of Roots in c.c.	Volume of Water exuded in c.c.
Urtica urens	1,350	3,025
Helianthus annuus ..	3,370	5,830

It has already been seen that the composition of exuded sap varies considerably in different plants. In many, e.g. potato, it is mainly composed of mineral salts, in others organic matter is present in large amount, as much as 8·8 per cent in Agave.

Industrial uses are made of this exudation of sap. Maple sugar is obtained in Canada by boring holes in the trunk of the sugar maple tree in the spring when the sap is rising. There is copious exudation of sap through these holes, and this is collected and evaporated down. In South America the natives prepare a special alcoholic drink from the sap obtained from Agave. This plant flowers but once in its life and then dies. Before flowering, a huge terminal bud is formed to the large inflorescence, the latter may often be as high as 30 feet and 8 inches in diameter. When the bud appears, a basin-shaped hollow is made in it, and the exuded sap collects here at the rate of about 5 or 6 litres per day. The exudate is collected, mixed with milk and allowed to undergo fermentation, the resulting liquor is called "Pulque."

Bleeding is a vital phenomenon and is markedly affected by external conditions. There must be an abundance of moisture in the soil and the temperature must be suitable. At 0° C. few plants show signs of bleeding. There must also be an adequate supply of oxygen to the roots or bleeding will cease in a short time, and it is also brought to an end by anaesthetics such as chloroform.

Mechanism of Exudation.—The exact mechanism of bleeding is still not understood. Pfeffer, at the time of his classical investigations on osmotic pressure, formulated three hypotheses to account for the exudation of fluid from living cells: (*a*) that the plasma-membrane develops unequal osmotic pressures in different parts of the cell; (*b*) that there is an unequal distribution of osmotic material in different parts of the cell; (*c*) that the osmotic material is present in the cell wall outside the membrane so that water is sucked out of the cell. The first hypothesis is obviously unsatisfactory, since it makes the osmotic pressure a function of the membrane instead of the concentration of the solute. The permeability of the membrane can only indirectly affect the osmotic pressure of the solution it encloses, by controlling through

exosmosis the concentration of the solution. Any osmotic pressure developed by a leaky membrane of this nature will depend in part only on the specific permeability of the membrane. The time during which exosmosis has continued, and the original concentration and mass of the solute, will be important factors bearing on the subject. If the membrane be rigid, the first pressure developed will be practically the same as if the membrane were truly semi-permeable.

Pfeffer advanced a scheme to account for bleeding, which is more in accordance with the known facts than some others that have been put forward to explain this phenomenon. In this scheme, the osmotic substance is supposed to exist in the cell at two different concentrations. Suppose a curved glass tube is closed by two completely semi-permeable membranes, A and B. The arm A is filled with a strong solution of cane sugar (say M/1), the arm above B with a weak cane sugar solution (say M/10); the intervening space is filled with water. Water will enter through A and through B, a pressure will be developed in the tube as a result, and when this pressure reaches the same value as the osmotic pressure of the solution above B, absorption above B will stop, since, owing to the pressure, the solution on one side of the membrane B will be in equilibrium with water on the other side. At A, however, there will be no equilibrium at the two faces of the membrane owing to the higher concentration of the solution, and water will therefore continue to enter. As a result the pressure in the tube will still continue to increase, and there will no longer be any equilibrium at B, but water will be forced out, and the solution in that arm will become more concentrated. If B were not immersed in water but in air, drops of water would appear at its surface. A transference of water has occurred through the tube, and so work has been done; but there is no contravention of the second law of thermo-dynamics, for the process will continue indefinitely as the two solutions will gradually mix, and the work has been done at the expense of the energy of diffusion.

Guttation.—The term guttation has been coined to cover the exudation of liquid from the uninjured parts of plants. It generally occurs in leaves at the tips of the veins, where there are special structures for outlet of liquid termed hydathodes. The passage of fluid from nectaries and digestive glands comes under the heading of guttation.

Guttation was first recorded in 1869 by de Bary who observed drops of water on the edge of leaves of the garden nasturtium, *Tropaeolum majus*. Normal guttation is best observed in the tropics where there is an abundant supply of water in the soil and the air is nearly always saturated with water vapour. In the aroids, guttation is large. As much as 110 c.c. per day has been recorded from the leaf of *Colocasia nymphaeifolia*. The water here is forced out near the tip of the leaf and the ejected drops follow one another in rapid succession.

Water losses from guttation take place under the same external conditions as bleeding, i.e. when there is an abundant supply of water in the soil and normal transpiration is checked by a humid atmosphere. Cool nights following warm days provide the best conditions for this phenomenon. Absorption of water from the warm soil is still active and transpiration has fallen almost to zero.

In the majority of cases, water of guttation escapes from the plant through more or less specialized organs, the hydathodes. Hydathodes occur mainly on leaves and vary in structure from the simple hairs found in Fuchsia to the complex organ of Primula. A number of fungal sporangiophores exhibit guttation and this is specially well shown in the case of Pilobulus.

In all cases of guttation, the water excreted is not pure but contains a small amount of other substances. In *Cicer arietinum*, *Circaea lutetiana* and *Epilobium hirsutum*, an acid liquid is excreted from the hairs on the leaves. The solution excreted from the sporangiophore of Pilobulus contains oxalic acid and sugars.

The mechanism of guttation is not known. The action of nectaries, for example, which excrete sugary solutions on their surface is obscure. It is simple enough to see how sugar placed on the exterior of a cell can withdraw water from the interior. The familiar experiment with the potato illustrates this well. If the top of a potato tuber be peeled and hollowed out into a basin-shape, and a strong solution of sugar be placed in this hollow, it will be found that after a time so much water has been withdrawn from the cells of the tissue that the solution has now overflowed down the sides of the tuber. It is a question of osmosis, and water passing from a weaker solution to a stronger one until equilibrium is reached. In nectaries, however, we have the reverse situation. It is possible that the matter may have something to do with the nature of the membrane, but at present we have no idea of the mechanism involved.

CHAPTER VI

THE ASCENT OF SAP

THE mechanism involved in the transport of water from the roots to the leaves of plants is still unsolved. In some plants, such as the Blue Gums of Australia and *Sequoia gigantea*, which are often over 400 feet high, the problem of water transport to the uppermost parts of the plants is obviously a serious one.

Although we are as yet ignorant of the exact mechanism of the ascent of sap, it has been known for a long number of years that the path of the water current is through the lumen of the xylem. Stephen Hales first showed that the xylem was the channel of transport of water from roots to leaves, and this work has been repeatedly confirmed by other workers. That water is conveyed through the wood, and not through the living tissues, can be demonstrated by placing cut shoots in weak eosin solution when the dye will be found to have stained the xylem. The "ringing" of a stem by removing a girdle of bark from the woody stem does not interfere with the upward transport of water, and such "ringed" plants will continue to transpire for a considerable time, while water plants which transpire but little, have only weakly developed xylem tissues. In 1885 it was shown by Kohl that if a stem were crushed with a compression screw, wilting of the leaves occurred, and when this pressure was removed, water passed up normally and the leaves recovered.

Sachs was one of the first investigators to put forward an explanation of the ascent of sap. He strongly held the view that the water passed up the walls of the xylem by the process of imbibition. This view has since been shown to be untenable.

The water passes through the lumen of the xylem. Elfving, Vesque and Errera employed the method of blocking the lower ends of cut shoots with cocoa-butter, and found that when the shoots were then placed in water, the leaves wilted. This result was confirmed by Dixon and Joly, who employed weak solutions of gelatin and low-melting paraffin wax for this purpose. They did find, however, that when paraffin was used, a small amount of water was able to pass up the walls of the xylem by imbibition, but the quantity taken up in this way was quite inadequate for the needs of the plant. The main mass of ascending water

passes through the outer annual rings and not through the central core.

What is primarily required is a dynamic and not a static explanation of the process: in other words, an explanation that will adequately account for the continuous drive of water up the cavities of the xylem. It has already been stated that some trees are as much as 400 feet in height, and the question arises, How is water driven up this great distance against the force of gravity? Various forces have been suggested to account for this ascent of water, such as capillarity and atmospheric pressure. But neither atmospheric pressure nor capillarity in themselves are sufficient. The highest column attained by capillary forces in narrow vessels of diameter 0·03 mm., is less than 4 feet, and the resistance encountered by the menisci of the capillary columns from the walls of the wood would also be very great. The maximum effect of atmospheric pressure would be to support a column of water 34 feet in height, when equilibrium would set in, so that atmospheric pressure may be ruled out as an explanation. In any case, both capillarity and atmospheric pressure are static processes and will not account for the continuous drive that is required.

Root-pressure was at one time considered to have an important bearing on the ascent of water in plants. It is true that this is a dynamic process, but in itself it is not sufficient to drive water a distance of 400 feet in the trunks of trees. For example, if water is to be raised to the height of 75 metres in *Abies pectinata* by root-pressure, a pressure of 7·5 atmospheres at least would be needed. Now bleeding in the Coniferae is very feeble, and was at one period entirely denied in these plants. As a matter of fact, a small amount of bleeding does occur in the Coniferae. Moreover, when transpiration is at its maximum in the summer, root-pressure is at a minimum.

It is therefore not surprising that the older investigators fell back on vital theories to account for the ascent of sap in trees. It was considered that the xylem parenchyma had an important bearing on the matter and exerted some kind of pumping action. The so-called "clambering" theory, put forward in 1884 by Godlewski, had a considerable amount of support at one period. According to Godlewski, there was some kind of periodic change in the osmotic pressure of the xylem parenchyma and medullary rays which brought about a pumping action and drove water

up the plant stem. By means of rhythmic changes in osmotic pressure, a parenchymatous cell would first of all draw water out of one trachea below it and then inject the water into another trachea above it. In this way a sort of staircase movement of water took place up the stem of the plant.

If this theory were true, then when the plant stem was killed the rise of water should cease. It was shown by Strasburger, in a series of experiments, that water will still continue to rise in plant stems when the living cells have been killed.

Strasburger made use of a number of trees and climbers. For example, he killed the stem of a Wistaria some 50 feet in height for a distance of 12 metres. The stem was then cut under eosin solution, and it was found that the dye was conveyed through the xylem over a distance of 10·5 metres in five days. A very similar experiment was performed with a hop plant whose stem was killed for a distance of 10·5 metres, and it was then found that a solution of dye was conveyed for a distance of 9·5 metres. In yet another experiment, an oak tree over 60 feet in height was cut, and the cut end placed in a tub of picric acid solution. The acid was taken up the stem for three days. The stem was next treated with a solution of fuchsin, when it was found that the dye was taken up for six days. The trunk was split down the centre, and it was discovered that the picric acid had been drawn up the wood for a distance of 15 metres, and the fuchsin for a distance of 21·8 metres, and this in spite of the fact that the acid must have killed the living cells of the trunk.

Dixon in his monograph, *Transpiration and the Ascent of Sap*, quotes some experiments of the Frenchman Boucherie, who cut trunks of trees and placed them in solutions of different poisonous substances. He found in every case that the solutions were drawn up to the uppermost portions, and he also showed that a second portion could be drawn up in the same way.

It has been pointed out that it is difficult to make water pass up a dead stem, and that the leaves quickly wilt and die when a portion of the stem has been killed. This result has been shown by Dixon to be due to the formation of toxic substances in the process of killing. In an experiment on *Prunus Cerasus*, in which he killed a portion of the stem by means of steam, he was able to wash out the toxic substances formed during the killing of the stem, and when these were removed, the upper leaves of the plant lived for twenty-one days.

The structure of the wood is also against any view of the ascent of sap being due to the pumping action of living cells. If the xylem parenchyma did perform some kind of pumping action, the cells should be arranged as shown in Fig. 19 to give the most efficient result, and not at the side of the vessels as they actually occur. Moreover, it has been shown by Strasburger, Dixon and others that water is able to travel in either direction in the stem. For example, Strasburger showed that in a pair of trees whose stems had organically fused, when the limb of one was cut below the region of fusion in such a way that the leaves of the severed trunk below the region of fusion were dependent on water supply from the other trunk, the leaves in this region lived for a considerable time; and he concluded that there was upward ascent of water in one trunk, and downward movement into the cut limb of the other tree. Dixon has shown that if the tip of an upper leaf of a potato plant be cut under a solution of eosin, the dye solution is quickly drawn back into the trachae of the conducting tracts of the leaf, and eventually makes its way into the upper branches and leaves, and also down the main stem. This passage of water in either direction in the stem would be quite impossible if there were some kind of pumping activity involved in the process, for there are no valves in the wood.

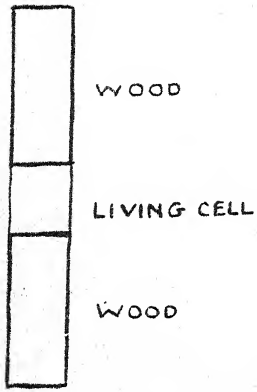

Fig. 19.

COHESION THEORY OF ASCENT OF SAP

The theory originally promulgated by Dixon and Joly in the 'nineties, that the ascent of sap was due to the cohesion of the water columns in the cavities of the xylem, and was drawn off from above by the leaf, has been put forward with great wealth of detail by Dixon in his monographs *Transpiration and the Ascent of Sap* and *The Transpiration Stream*. The following is a summary of these investigations.

The cohesion theory of the ascent of sap is at present the most satisfactory attempt that has been made to explain the ascent of

water in plants. The cohesion theory as put forward by Dixon and Joly, and supported by a large mass of qualitative and quantitative data, has tended to overshadow all other work on the subject.

It has been known for a long time that water possesses strong

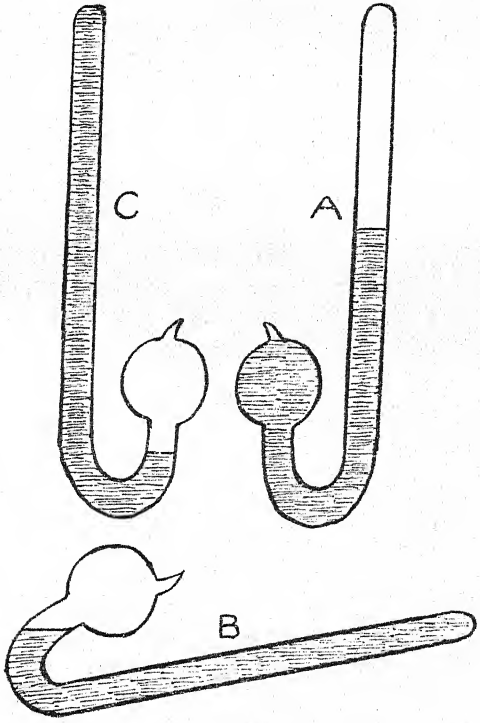

FIG. 20.—The "water-hammar."

cohesive properties. This can be readily demonstrated by means of the so-called "water-hammar" (Fig. 20). If a glass tube be bent in the manner shown in Fig. 20, and the bulb at the lower end filled with water, and a vacuum created within the tube by boiling the water and sealing off the end, it can be shown by suitable adjustment from position A to position B, and finally to position C, that the forces of adhesion between glass and water, and the cohesion of the water molecules, are such that the column of water will hang down against the gravitation force

of the entire water column. The column, however, is unstable, and if a bubble were to form immediate collapse would occur.

It was shown in 1847 by Donny that it is possible to support a column of sulphuric acid 1·255 metres high in a vertical tube, sealed at the upper end, when atmospheric pressure is not allowed to press the liquid upwards from below. Berthelot has found that water has a high cohesive force and can withstand tensile strains. He demonstrated this cohesive force of water by sealing one end of a strong capillary tube and drawing out the other end into a fine point. The tube was then filled with water, heated to 28° to 30° C., and cooled to 18° C., when a little air was drawn through the fine opening of the drawn-out end. This fine point was then sealed off. The tube was now heated once more to 28° to 30° C. In the course of the heating the bubble of air disappeared, and when the tube was cooled to 18° C. did not reappear; the water still completely filled the tube, adhering strongly to the glass. Berthelot calculated from the expansion produced by heating the tube, that a force of nearly 50 atmospheres would have to be exerted to produce a similar result in the opposite direction. Dixon has since estimated that freshly expressed sap, from which the dissolved gases have not been removed, shows a cohesion that is only overcome by the exertion of a tension of over 200 atmospheres, while Ursprung and Renner consider that this value should probably be raised to 315 atmospheres.

According to Dixon and Joly, the ascent of sap takes place in the following way: The water in the conducting channels (vessels and tracheids) hangs in fine columns, a film of water adhering to the walls of the wood, and the remaining mass of liquid cohering to the film. From this initial stage we can go a step farther and consider the passage of water from the root-hairs through the stem to the leaves. By means of the suction pressure of the root-hairs and cortical tissue, water is withdrawn from the soil, and enters the xylem (see Chapter III). By means of adhesion and cohesion, the water hangs in the xylem strands in fine columns and from these columns, which are in a high state of tensile strain, it is withdrawn from above by the mesophyll cells of the leaf, and by subsequent evaporation from the intercellular spaces, passes into the external atmosphere.

These hanging columns of water can exert a pull equal to that of a fine steel wire, but they can only do this while they are

attached to the walls of the containing tracheae. The initial force in the process is exerted by the root.

The question arises, What tensions are required for this process? What forces are necessary to draw water through the xylem to make up the losses from transpiration in the leaf? Dixon has found that the resistance of the wood to the water column is not as great as Ewart considered, and that, even in the tallest trees, this is small compared with the tensile strength of water. Dixon has shown that water may be moved through a stem in a horizontal direction at the same velocity as the transpiration current if urged by a head of water equal to the length of the stem. In the case of a vertical stem, twice the head will be needed to urge water in the upward direction at the same speed as the transpiration current.

It has already been seen that, on the cohesion theory, the water in the conducting tracts of the wood hangs in the form of unbroken columns, and that these columns are continuous one with the other, both in the vertical and lateral direction through the cell walls. There is in reality a meshwork of water throughout the plant, extending from the roots to the leaves. According to Dixon and Joly, the termini of this water network are the menisci of the water in the submicroscopical cavities of the cell walls of the mesophyll cells bordering upon the intercellular spaces in the leaf and the epidermal cells of the root. Now these cells in the leaf will evaporate water from their walls, and the water molecules will escape through the submicroscopical cavities into the intercellular spaces, and from thence into the outside air. As a result of the imbibitional forces in the evaporating walls of the leaf cells, the water in the conducting tracts of the xylem will be thrown into a state of tension. In the course of transpiration, water molecules will be set free from the cavities in the walls of the leaf cells, and fresh water will be abstracted from the protoplasm of the cells lining the walls. The protoplasm in turn will draw upon the cell vacuole, and eventually the water in the xylem tracts of the leaf will be called upon as a source of supply. Thus a pull is set on the water in the petiole xylem channels, and this pull is carried down the petiole to the stem, and finally to root and soil.

Although the cohesion theory of the ascent of sap explains in a remarkable way a large number of facts that have been observed about the transpiration stream, in actual practice it is difficult

to devise crucial experiments that would definitely settle the truth or otherwise of the theory. From the Dixon point of view the matter is reversible, that is to say, the plant should react equally well if it were placed upside down. It must be remembered, however, that it would be quite impossible in practice to obtain the same contact between the soil particles and the leaves as exists between the root-hairs and the soil.

One of the main criticisms that has been brought against the cohesion theory is the presence of air-bubbles in the wood. It has been shown by Dixon that dissolved air does not affect the tensile strength of water, but the presence of free bubbles of air in the hanging water columns of the wood would destroy their stability and bring about their collapse. It is impossible to state definitely whether air-bubbles seen in cut sections are brought there by mechanical manipulation, or whether their presence is natural in the wood of the living plant. It was stated by Holle* that in leaves that had been allowed to wilt completely, the water columns were still intact in the xylem when examined under the microscope. Bode† has taken up a similar standpoint, and claimed that under ordinary conditions there are no air-bubbles in the xylem even in the very last stages of wilting.

Dixon has accepted the presence of bubbles in the wood, but the difficulty is to get rid of them. The suggestion has been made that perhaps root-pressure in the spring is sufficiently strong to remove them. But when the whole plant is transpiring rapidly, a negative pressure is set up in the wood, and the bubbles are then under a negative pressure; when a positive pressure is introduced they will suffer compression. In any case, in tall trees such as the Blue Gums of Australia, root-pressure alone would be inadequate to remove any air-bubbles that may have formed.

The most cogent reason that has been urged against the cohesion theory is the fact that tracheids should be more efficient than vessels. In Dixon's own words: "The salient feature of the wood is the subdivision of the waterways by an immense amount of transverse and longitudinal divisions into minute compartments . . . the vessels and tracheids. For the system the function of which is the conducting of water this is evidently the most unexpected configuration. It is true that the partitions are permeable to water; but when a considerable distance has

* *Flora*, 1915, **108**, 73. *Jahrb. f. wiss. Bot.*, 1923, **62**, 92.

to be traversed the sum of the distances opposed by the walls becomes very important. . . . It is evident from the persistence of walls in the development of the water-conduits of a plant, introducing as they do an immense resistance to flow, is inexplicable on any view that regards water being forced through the stem by some unknown force. The cohesion theory, on the other hand, gives a ready explanation, for it confers stability on the tensilely stressed transpiration stream." From this statement it is evident that tracheids with their numerous short compartments would give greater stability to a tensilely stressed stream of water than vessels in which the cross-partitions are relatively far apart. Such being the case, it is a curious anomaly that our present dominant flora, the Angiosperms, have practically without exception adopted vessels in the place of tracheids.

It must be admitted that at the present time there is no adequate explanation of the ascent of sap, and although the cohesion of water possibly plays a considerable part in the matter it is probably only a portion of the truth of the whole process.

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PART II

METABOLISM

CHAPTER VII

CATALYSIS AND ENZYMES

It is a well-known fact of chemistry that the rate of a reaction may be greatly increased by the presence of a third substance. Cane sugar is slowly hydrolysed by water alone, but the rate of the reaction is enormously increased by the presence of acid. Similarly, when potassium chlorate is heated alone, it melts and slowly splits up into potassium chloride and oxygen; but the addition of a small amount of manganese dioxide brings about the reaction at a temperature below the melting-point of the chlorate, and the oxygen is rapidly and smoothly evolved. The preparation of sulphur trioxide from sulphur dioxide and oxygen needs the presence of spongy platinum. In all the reactions described above, the presence of a third substance has increased the rate of the reaction, and the addition product appears at the end of the reaction unchanged and may be used over and over again. Such reactions are termed *catalytic reactions* (κατα = down and λυο = to loosen, eventually coming to mean to hasten), and the substance which exerts the catalytic effect or accelerates the reaction is termed a *catalyst* or *catalysor*. These terms were first introduced in 1837 by Berzelius.

Ostwald has defined a catalyst as a substance which alters the velocity of a reaction, but does not appear in the end products. Until recently, it was considered that a catalyst could not initiate a reaction, but only accelerate one that was already proceeding. Take, for example, the reaction between hydrogen and oxygen to form water. If finely divided platinum be added to the mixture, the reaction proceeds with explosive violence at ordinary temperatures. The mixed gases left at ordinary temperatures will keep indefinitely. It might therefore appear that the platinum initiates the reaction. But when the gases are heated to 440° C., they will combine with measurable velocity, and at lower temperatures still they will combine after prolonged heating. Since the rate of the reaction falls with temperature, it can be understood that the rate of combination is so slow at ordinary temperature as not to be measurable.

The alternative view to that given above is that a catalyst can initiate a reaction. The catalyst is looked upon as a source of

surface energy, the chemical nature of the catalyst being relatively unimportant, so long as the space configuration of the atoms on the surface of the catalytic reagent are such as to cause certain orientated adsorption relationships, and the surface of the catalyst is in such a condition as to contribute a given quantity of surface energy to the system. The view that a catalytic reagent is able to initiate a reaction removes the old difficulty of explaining how substances of very different chemical nature could be used to catalyse one and the same chemical reaction. On this newer view, however, as long as these various chemical substances are able to contribute equal amounts of surface energy, and orientated adsorption is considered important, it is possible to explain (*a*) that different substances in a suitable physical condition are able to accelerate the same reaction and (*b*) are able to initiate a reaction not already in progress. A catalyst will be able to initiate a reaction by contributing to the system the requisite amount of surface energy which is necessary to start the reaction.

A catalyst, therefore, is a substance that is able to alter the rate of a reaction either in the direction of acceleration or of retardation, and may in certain cases even initiate a reaction not already in progress.

The catalyst is usually present in relatively small concentration. This is connected with the fact that it is not used up in the course of the reaction, so that a relatively small concentration of catalyst is able to effect the transformation of a large amount of the reacting substance.

Living organisms make wide use of catalysts in various cellular reactions. Berzelius was the first investigator to draw attention to this fact. It has been known from prehistoric times that fruit juices allowed to stand without special precautions give rise to ethyl alcohol and evolve carbon dioxide, hence the term "fermentation" (*fevere* = to boil). The work of Pasteur showed that the production of wine and other spiritous liquors, and the leavening of bread, were brought about by the action of various micro-organisms, for when these products were kept under sterile conditions the reactions did not take place. Prior to Pasteur's discovery, various substances had been isolated from living organisms, and, from the similarity of their behaviour to alcoholic fermentation, they came to be called "ferments." For example, in 1833, Payen and Persoz isolated from malt, by the addition

of alcohol, a product which could be dried and preserved, and which was able to hydrolyse starch, and to which they gave the name "diastase." When, however, it was shown by Pasteur that alcoholic fermentation was due to the presence of a living organism, two types of ferments were distinguished, "organized ferments" and "soluble" or "unorganized ferments." The unorganized ferments were the products which could be made to produce their reactions apart from the living organism, e.g. diastase. This double use of the term ferment led to very considerable confusion, and in 1878 Kühne suggested the name *enzyme* (ἐν ζύμῃ = in yeast or in leaven) in place of unorganized ferment, and this term has now been universally adopted to designate these biological catalysts.

E. F. Armstrong defined enzymes as "selective colloidal catalysts, present in living cells and destroyed by heat." The late Sir William Bayliss objected to this definition on the grounds of the use of the words "selective," "colloidal" and "temperature." For all the reasons given by Bayliss for his objection to Armstrong's definition, the reader must be referred to his monograph on enzymes, *The Nature of Enzyme Action* (especially Chapter 1, p. 12). Haldane, more recently, has defined enzymes as "soluble, colloidal, organic catalysts produced by a living organism." Presumably Bayliss would also disagree with this definition. Yet another definition of an enzyme has been given by Waldschmidt-Leitz, who considers them to be "definite material catalysors of organic nature with specific powers of reaction, formed indeed by living cells, but independent of the presence of the latter in their operation." This is a fairly satisfactory definition and is to be preferred to either that of Armstrong or Haldane, as it does not commit itself to any special physical nature of enzymes.

The substance upon which an enzyme acts is termed the *substrate*. Duclaux suggested that the termination *-ase* be taken as denoting an enzyme which acts upon a particular substrate, i.e. maltase for the enzyme which hydrolyses maltose, lactase for the enzyme which hydrolyses lactose, and so forth. There are, however, certain enzymes which have been known for so long that their original names have been retained, e.g. pepsin and trypsin.

A large number of enzymes have now been isolated from living organisms and their reactions studied. Their use to the living

organism is of the utmost importance. Thus, for example, the metabolic products of plants, such as starch, proteins and fats would be quite useless if they were not brought into a suitable state for assimilation and translocation. Different enzymes act upon these different substrates, and the living plant is able to utilize the products of these reactions in its various physiological activities.

Enzymes may be secreted by living organisms and act outside the protoplasmic mass. Examples of such extracellular enzymes are ptyalin, the starch-hydrolysing enzyme of saliva, pepsin, protease of gastric juice, and invertase (sucrase) of yeast, which hydrolyses cane sugar to glucose and fructose. Intracellular enzymes appear to be non-diffusible through the cell membrane. These intracellular enzymes carry out their reactions within the cell, and in some cases it is possible to destroy the protoplasm without inactivating the enzyme.

CLASSIFICATION OF ENZYMES

Enzymes are best classified according to the reactions they catalyse. The greater number of enzymes are concerned with hydrolysis of various substrates, so that the elements of water enter into the reactions, and these reactions usually take place in the presence of an excess of water. A list is given below of the more important enzymes, and the specific reactions in which they take part will be considered in greater detail under the different chapters of metabolism.

(I) HYDROLYSING ENZYMES

Enzyme	*Substrate*	*End-product*
(a) Enzymes hydrolysing Esters		
Lipase	Fats	Higher fatty acids + glycerol
Butyrase	Lower esters	Lower fatty acids + alcohols
Chlorphyllase	Chlorophyll *a*	Chlorophyllide *a* + phytol
Tannase	Tannin	Glucose + gallic acid
Pectase	Pectin	Pectic acid + methyl alcohol
Phytase	Phytin	Inositol + phosphoric acid
Sulphatase	Phenol sulphates	Phenol + potassium hydrogen sulphate
Lecithinase	Lecithin	Choline + fat + phosphoric acid

Enzyme	*Substrate*	*End-Product*
(*b*) *Enzymes hydrolysing Carbohydrates.*		
(i) *Polysaccharidases.*		
Cellulase	Cellulose	Cellobiose
Cytase	Hemicellulose	Simple sugars
α-Amylase	Soluble starch	α-maltose
β-Amylase	Soluble starch	β-maltose
Inulase	Inulin	Fructose
Pectinase	Pectic acid	Galactose+galacturonic acid
(ii) *Trisaccharidases.*		
Raffinase	Raffinose	Melibiose + fructose
Gentiobiase	Gentianose	Gentiobiose + glucose
(iii) *Disaccharidases.*		
Invertase (sucrase)	Sucrose	Glucose + fructose
Maltase	Maltose	Glucose
Lactase	Lactose	Glucose + Galactose
(*c*) *Enzymes hydrolysing Glycosides.*		
(i) *α-Glycosidases.*		
Maltase	α-*d*-Glycosides	Glucose + non-sugar
Trehalase	Trehalose	Glucose
(ii) *β-Glycosides.*		
Emulsin (mixture)	All β-Glycosides	Sugar + non-sugar residue
Amygdalase	Amygdalin	Glucose + prunasin
Prunase	Prunasin	Glucose + *d*-mandelonitrile
Myrosinase	Sinigrin (myrosin)	Glucose + allyl isothiocyanate + potassium hydrogen sulphate
(*d*) *Enzymes hydrolysing Nitrogen Compounds.*		
(i) *Proteases.*		
Rennin	Casein	Paracasein
Pepsin	Native proteins	Proteoses and peptones
Trypsin	Native proteins	Polypeptides and amino-acids
Erepsin	Polypeptides	Amino-acids
Papain	Native proteins	Polypeptides and dipeptides
Bromelin	Native proteins	Polypeptides and dipeptides
(ii) *Desamidases*		
Urease	Urea	Carbon dioxide + ammonia
Arginase	Arginine	Urea + ornithine
Histozyme	Hippuric acid	Benzoic acid + glycocoll

(II) DESMOLYSING ENZYMES

Enzyme	*Substrate*	*End-product*
Zymase (mixture?)	Hexose	Ethyl alcohol + carbon dioxide
Glycolase	Hexose	Lactic acid
Decarboxylase	R . CO . COOH	R . CHO + CO_2

(*a*) *Enzymes involved in Oxidation-reduction.*

Alcoholoxidase	Ethyl alcohol	Acetaldehyde
Purinoxidases	Hypoxanthine	Xanthine
	Xanthine	Uric acid
	Uric acid	Allantoin + carbon dioxide
Glyoxalase	Methylglyoxal	Lactic acid
Reductases	Methylene blue	Leuco base
Phenolases	Phenols	Quinones
Laccase	Lac	Lacquer
Tyrosinase	Tyrosine	Melanin
Peroxidase	Peroxides	Active oxygen + red coloured product
(*b*) Catalase	Hydrogen peroxide	Water + oxygen

METHODS OF ISOLATION AND PURIFICATION OF ENZYMES

As the greater majority of enzymes are intracellular, a variety of different means have been employed to isolate them from tissues. Many cannot be isolated without disintegration of the tissues by grinding with sand or kieselguhr, or the tissues can be allowed to disintegrate by autolysis. In some cases, it is possible to obtain the enzyme in aqueous solution by extraction of the tissue with water or weak glycerol solution that has been saturated with either toluene or chloroform. Apparently the plasma-membrane is rendered sufficiently permeable to allow of the diffusion out of the enzyme. Enzymes in many cases may be precipitated from their aqueous solution as amorphous powders by the addition of alcohol.

An enzyme isolated from tissues in the various ways described above is always accompanied by foreign substances, chiefly proteins, carbohydrates and inorganic salts. Different methods of purification have to be employed for different enzymes. One of the most widely applicable methods of purification is adsorption of the enzyme on different surfaces. This method has been much developed by Willstätter and his co-workers. The adsorption method of purification is especially useful in separating one enzyme from another. It is based upon the fact that a variety

of adsorbents remove enzymes from solutions, and the enzyme may then be eluted from the adsorbent by a change in the pH, which will bring about a change in the nature of the charge on the enzyme or adsorbent or on both. The most important adsorbents that have been used for purification are kaolin, aluminium hydroxide and charcoal.

THE CHEMICAL NATURE OF PURIFIED ENZYMES

Purification of enzymes is a matter of great difficulty. At one time they were considered to be proteins, a view mainly based on the principle that anything that one did not understand was classed as a protein. It is an odd fact that the greater number of successful attempts to purify enzymes have given substances which are predominantly non-protein. The purest preparations that have so far been obtained on analysis give C, H, O, and N and are non-dialysable, and give no protein reactions.

The only preparations of enzymes that can make any pretence of purity are urease, trypsin, pepsin, invertase (sucrase), lipase, xanthine-oxidase and peroxidase. Willstätter is the chief protagonist for the non-protein nature of enzymes, and his views will be considered in greater detail below; on the other hand, Sumner, as well as Northrop, maintains that certain enzymes, notably urease, pepsin, and trypsin are definitely proteins.

According to Willstätter, who with his co-workers has made a series of most determined assaults on the chemical nature of enzymes, "enzymes are composed of one or more specific active groups, and of one, or more, less unspecific, and therefore changeable, colloid bearers; the former is considered responsible for the specificity, and the latter primarily for the catalytic activity and for the stability of the active groups."

The colloid bearer or "Trager" being changeable, may be substituted by other colloids, and this gives the basis for the removal of the enzyme from its solution by alumina, kaolin, etc. Moreover, it has led to the view that enzymes are not proteins, but that the colloidal carriers may be in the natural condition of the enzyme in the living cell; and they can be replaced by other proteins or non-protein carriers.

In 1926 Sumner* was able to isolate from the Jack Bean a crystalline product which he claimed to be pure urease. The method of preparation is as follows: 100 gm. of finely ground

* *J. Biol. Chem.*, 1926, **69**, 435; **70**, 97; 1928, **76**, 149.

meal from the Jack Bean is treated for a few minutes with 500 c.c. of 31·6 per cent solution of acetone and the whole is then poured on a filter and left overnight in an ice-chest. Crystals will be found in the filtrate next morning. These may be readily re-crystallized. They are centrifuged and twice washed with 32·0 per cent solution of acetone. They are then dissolved in a small amount of water, and centrifuged once more to remove suspended matter. Enough acetone is added to make a 32·0 per cent solution, and the mixture is placed in the ice-chest once more and a phosphate buffer of pH 5·9–6·1 in 32 per cent acetone added very gradually. Sharply defined octahedra crystals will be thrown down.

The urease isolated in this manner is apparently a globulin (see Chapter X) and is easily soluble in water. Water carefully purified by distillation has no immediate destructive effect on this product, and any destructive effect of water is due to the presence of traces of heavy metals. The crystals isolated by Sumner were found to be 8,400 times more active than their weight of ordinary soy bean meal. This purified preparation is so powerful that it will hydrolyse a solution of urea at 20° C. at such a rate that 120 times its own weight of ammonia is produced every five minutes.

Northrop* has now been able to prepare pepsin and trypsin in the crystalline state, and both these preparations are claimed to be proteins. Crystalline pepsin is obtained by salting out a sulphuric acid solution of the enzyme with saturated magnesium sulphate solution. The product is then dissolved in alkali, re-precipitated with acid, redissolved in warm alkali and allowed to cool slowly. Crystalline trypsin was isolated by the addition of saturated ammonium sulphate solution to a concentrated solution of trypsin in one-quarter saturated ammonium sulphate. Crystalline pepsin forms hexahedra, which tend to crystallize in clusters. Elementary analyses for urease, pepsin, trypsin, together with the figures obtained by Willstätter for peroxidase, are given below:

	(*Percentages*)
Crystalline Urease	C = 51·6; H = 7·1; N = 16·0; S = 1·2; Ash = 2·0
Crystalline Pepsin	C = 52·4; H = 6·66; N = 15·4; S = 0·85; P = 0·078; Cl = 0·21; Ash = 0·47

* *J. Gen. Physiol*, 1932, **16**, 267, 295, 313, 323, 339.

(*Percentages*)

Crystalline Trypsin	C = 50·0; H = 7·2; N = 14·9; S = 1·10; Cl = 2·88; Ash = 1·2
Peroxidase (ash-free)	C = 46·0–49·4; H = 6·9–8·6; N = 9·4–13·6; P present in traces and only about 0·1 Fe in purest preparations.

Northrop was unable to find any evidence for the existence of a mixture of active and inactive material in the crystals of pepsin he had isolated after recrystallization, by solubility determinations in a number of different solvents, inactivation by heat or alkali or by rate of diffusion. There are one of two possibilities here: either Northrop was dealing with a single substance or with a solid solution of two or more closely related substances. If he were dealing with a solid solution of two or more substances, the assumption must be made that they have all the same degree of solubility in different solvents and the same diffusion coefficient, and also that they are changed at the same rate and to the same extent by alkali. Further, that the conditions for reactivating the enzyme are the same as for reversing the denaturization of protein. These are difficult assumptions to justify in all the circumstances detailed above, and it is scarcely probable that every one of these conditions would apply to all the components of a mixture. If Northrop's product be a pure protein, then it follows that the enzyme is a protein. On the other hand, Willstätter has maintained that the crystalline product isolated by Sumner is a complex, and that the enzyme urease is present as an adsorbed impurity upon a crystallizable but inert globulin surface.

The divergent points of view of Willstätter on the one hand, and of Sumner and Northrop on the other, on the nature of enzymes is best summarized in Northrop's own words: "It does not necessarily follow even if the material represents the pure enzyme that it is the most active preparation that can be obtained, nor that it is the only compound that has proteolytic activity. It is possible that haemoglobin is the type of structure for the enzymes and that they consist of an active group combined with a protein as suggested by Pekelharing. The active group may be too unstable to exist alone, but it is quite conceivable that a series of compounds may exist containing varying numbers of active groups combined with the protein, and that the activity of the compound would depend on the number of these active groups. This hypothetical complex would not differ much from

that assumed by Willstätter and his co-workers, except that it supposes a definite chemical compound of the active compound with the protective group, in place of an adsorption complex." This seems a fair statement of the situation as we know it at present and certainly brings the two views into a fair amount of harmony.

KINETICS OF ENZYME ACTION

According to the Law of Mass Action, which has been briefly considered in Chapter II, the rate of a chemical reaction is proportional to the concentration of the reacting molecules, i.e. the gram-molecules per litre. Since the amount of unchanged substance is continually decreasing, the amount changed in unit time will be greater at the commencement of the reaction than towards the end. If we consider the case of the hydrolysis of maltose by acid to glucose, the rate of the hydrolysis will be proportional to the concentration of maltose present and to the amount of glucose that has been formed:

$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6$$

Now such a reaction takes place in the presence of an excess of water, and the change in the concentration in water during the course of the reaction may therefore be ignored. Such a reaction as this is termed a monomolecular reaction, and can be expressed by the equation:

$$V = \frac{dx}{dt} = K(a - x)$$

where V is the rate of the reaction at time t, x is the amount of substance which has already been transformed in time t, $(a - x)$ is the concentration of material which is being transformed, a being the initial concentration of the substance, and K is a constant.

It is, however, impossible to apply this equation directly to the experimental results, since dx, the amount of change of x in the time dt, would have to be taken fairly large in order to obtain accurate results, and during the interval $(a - x)$ would naturally have diminished. The difficulty is overcome by integrating the equation, when we obtain:

$$\frac{1}{t} \log_e \frac{a}{(a - x)} = K$$

as it is simpler to work with ordinary logarithms to the base 10, rather than with logarithms to the base e, we obtain for the equation:

$$\frac{1}{t}\log_{10}\frac{a}{(a-x)} = 0\cdot4343K$$

The value of K is characteristic for a given reaction at a given temperature, but changes if the conditions which affect the reaction or its mechanism are altered.

The hydrolysis of either cane sugar or maltose in the presence of acid and using the above equation gives a logarithmic curve

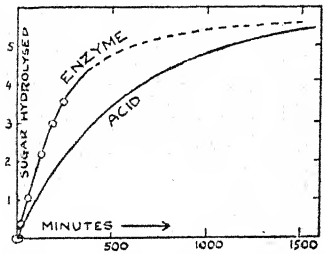


FIG. 21.—Rates of enzymic and acid hydrolysis of cane sugar in solutions of equal initial concentrations (5·7 units as measured by the polarimeter). Both reactions reach the same equilibrium point, but while the acid hydrolysis begins to slow off from the beginning, the enzyme action maintains an almost constant rate for about 4 hours and then slows off suddenly. (The "enzyme" curve is drawn and the "acid" curve calculated from Michaelis' data.) (From Parsons, *Fundamentals of Biochemistry*.)

(Fig. 21). If the course of hydrolysis of lactose by lactase be considered, the velocity-constant K shows a fall with time. Another case is trypsin. On the other hand in the hydrolysis of cane sugar by invertase the velocity-constant shows a rise with time. The figures for lactose hydrolysis by lactase are given below:

Time in Hours	*Velocity-Constant* (K)
1	0·0640
2	0·0543
3	0·0460
5	0·0310
24	0·0129

The question arises as to why the velocity-constant should show a fall. Two points must be borne in mind in this connection. In the first place, we have every reason to suppose that the reaction we are dealing with is reversible (see below). This being the case,

the observed difference in the rate between the forward and reverse reaction, with fall in the concentration of the substrate, will eventually tend to show the synthetic reaction more markedly than the hydrolytic one. But with *in vitro* experiments, the equilibrium point is very near that of complete hydrolysis, since there is an excess of water present, so that the influence of the synthetic reaction is small. It is therefore evident that some other cause must be at work to slow up the rate of hydrolysis.

The rate of a catalysed reaction is dependent on the concentration of the catalyst. If any factor should arise during the course of the reaction to inhibit the activity of the catalyst, the rate of the reaction will obviously fall. Enzymic solutions are very sensitive to changes in pH, and on keeping they tend to lose their activity; but the most adequate explanation of the course of a hydrolysis of a substrate catalysed by an enzyme, is the rapid formation of some kind of complex between substrate and enzyme, and that it is only the amount of substrate so combined that undergoes change. To make this matter clear, some investigations will be considered in further detail.

The action of amylase or diastase on starch was investigated by Horace Brown and Glendinning.* It was found that in the early stages of the reaction, equal amounts of starch were hydrolysed in equal times by the amylase. Thus a straight line and not a logarithmic curve was obtained (Fig. 21). As the reaction proceeded, however, the curve became truly logarithmic. In the initial stages of this reaction, the concentration of substrate relative to that of enzyme was very large, and so long as this excess of starch was present unhydrolysed, the amount of starch per unit volume was very large compared with the amount of starch combined with amylase. As long as there was this excess of the substrate present in the reaction mixture, the combination between enzyme and substrate would remain nearly constant in amount, and the result would be that equal amounts of starch would be hydrolysed in equal times and a straight line curve would be obtained. With time, as more and more starch is hydrolysed, the concentration of the substrate would show a heavy fall; and, as a result, the amount of combination between enzyme and starch as well as the rate of hydrolysis will follow the law of mass action more closely.

Adrian Brown† found, for the early stages of inversion of cane

* *Trans. Chem. Soc.*, 1902, **81**, 388.

† Ibid., 1902, **81**, 373.

sugar by invertase, that a straight line curve was obtained, that is to say in the initial stages of the reaction equal amounts of sugar were inverted in equal times, whereas according to the law of mass action these amounts should have been proportional to the concentration of cane sugar. Brown advanced the explanation for this result, that not only is there combination between enzyme and substrate, but that this complex is able to exist for an appreciable time, so that in a given interval of time a particular amount of enzyme is only able to bring about a limited number of complete molecular changes. Similarly, E. F. Armstrong,* who has investigated the hydrolysis of maltose and lactose by maltase and lactase respectively, found that the initial and end stages were linear, and he also assumed that combination occurred between enzyme and substrate. At the beginning of the reaction, excess of substrate was present, but at the close of the reaction, excess of enzyme, and both conditions favour the reaction taking a linear course.

Michaelis and Menten† have suggested that there is an intimate union between enzyme and substrate. This view is based upon the specific nature of enzyme reactions (see below). Thus invertase will only hydrolyse cane sugar, and lactase will only hydrolyse lactose, lactase having no influence upon cane sugar or invertase upon lactose. There is thus a specific relationship between substrate and enzyme. There is little doubt that the rate of chemical change is definitely linked up with the properties of the intermediate complex formed between enzyme and substrate and in simple cases this can be correlated with (*a*) the rate at which the complex is formed, (*b*) the rate at which the complex decomposes, and (*c*) the rate at which the decomposition products diffuse away from the enzyme surface.

According to Michaelis and Menten, the velocity of reaction will be proportional to the concentration of the complex, and this in turn will be related to the concentration of enzyme and of substrate. We thus have:

$$\text{Substrate} + \text{Enzyme} \rightleftharpoons \text{Complex}$$

It will simplify the problem if we consider a specific case, for example the hydrolysis of cane sugar by invertase:

$$S + E \rightleftharpoons SE$$

* *Proc. Roy. Soc.*, 1904, **73B**, 500.

† *Biochem. Zeit.*, 1913, **49**, 333.

The first stage of the reaction will be the formation of the complex SE, and the second stage of the reaction will be the decomposition of the complex into glucose and fructose:

$$SE + H_2O \rightarrow E + G + F$$

Now if e represents the total molar concentration of enzyme, s that of the substrate, and if the concentration of substrate be much greater than e, and if p represents the concentration of substrate-enzyme complex molecules, the concentration of enzyme molecules will be $(e - p)$. Then if K_m represents the dissociation constant of the complex SE, we have according to the law of mass action:

$$K_m p = (e - p)s$$

or

$$K_m = \frac{(e - p)s}{p}$$

if k be the velocity-constant of the break up of SE and if v represents the instantaneous velocity of reaction, i.e. $\frac{ds}{dt}$ of hydrolysis, and the amount of water is practically constant:

$$v = kp = \frac{kes}{K_m + s}$$

or if V be the velocity when s is large compared with K_m:

$$v = \frac{Vs}{K_m + s} \quad \text{or} \quad K_m = \left(\frac{V}{v} - 1\right)s$$

K_m is termed the "Michaelis constant" and is characteristic of the enzyme, and V is proportional to its concentration. Plotted graphically the above equation gives a rectangular hyperbola.

The assumption underlying the Michaelis and Menten's view of combination between enzyme and substrate is that the velocities of the formation and dissociation of the complex are infinite when compared with the velocity of decomposition of the complex to form the products of the reaction. As Haldane has pointed out (*Enzymes*, Chapter III, p. 40) this is a somewhat improbable assumption. It means that the combination of enzyme and substrate is always in equilibrium. For example in a 0·1N solu-

tion of cane sugar only 10^7 sugar molecules will be able to collide with a given point on the surface of the enzyme invertase per second, "and we cannot suppose that the orientation would always permit of union with the enzyme."

G. E. Briggs and Haldane* have therefore re-examined the theoretical basis of the Michaelis and Menten equation in the light of this criticism. If we take the case of some molecule A, which is irreversibly converted to a product B, and consider this reaction to be catalysed by an enzyme, and further suppose that it is unimolecular as regards A, we shall have:

$$A + E \rightarrow AE,\ AE \rightarrow A + E,\ AE \rightarrow B + E$$

if k_1, k_2 and k_3 represent the velocity-constants of these three reactions and e and p having the same significance as before, then:

$$\frac{dp}{dt} = k_1 s(e - p) - k_2 p - k_3 p$$

So long as the velocity of reaction is constant p will be constant, but even when the rate is changing, the change in p will be infinitesimally small compared with the rate of change of s, hence $\frac{dp}{dt} = 0$

$$\therefore\ k_1 s(e - p) = p(k_2 + k_3)$$

$$\therefore\ p = \frac{k_1 es}{k_1 s + k_2 + k_3}$$

$$= \frac{es}{s + \frac{k_2 + k_3}{k_1}}$$

Thus if we take $K_m = \frac{k_2 + k_3}{k_1}$ the result will be the same as that of Michaelis and Menten.

TEMPERATURE

Temperature has a twofold action upon enzyme reactions. In the first place, rise in temperature speeds up the rate of the reaction, and in the second place, rise in temperature has a destructive effect upon the enzyme. It was at one time supposed

* *Biochem. J.*, 1925, **19**, 338.

that each enzyme had a characteristic optimum temperature, but the optimum point is influenced by a variety of factors other than the nature of the enzyme itself. Thus the concentration of the enzyme, and the nature and concentration of the substrate upon which it is acting, as well as the pH of the medium in which the enzyme is dissolved, all enter into the problem. There is therefore a good deal of uncertainty as to whether the observed differences in temperature optimum are really due to specific differences between the enzymes, or whether they are due to various factors attending their action. In the great majority of cases the temperature optimum for enzymes lies between 40–45° C. The plant proteolytic enzymes, bromelin and papain, are exceptions to this rule and have a temperature optimum of 60° C.

The time factor is also of importance in this connection. There can be no optimum of either temperature or of hydrogen ion concentration independent of time. The auto-inactivation of an enzyme which proceeds slowly at lower temperatures is increased by higher temperatures until it balances the accelerating effect of temperature rise. Above the optimum point, further increase in temperature leads to rapid inactivation.

The temperature coefficient Q_{10} has been studied for a number of enzymes. The data available at present are very unreliable and conflicting. Most of the values that have been obtained are below 2·0, i.e. less than for the majority of chemical reactions, but in some cases, notably trypsin, higher values than 2·0 have been recorded.

HYDROGEN-ION CONCENTRATION

Enzyme preparations as a general rule exhibit a definite optimum hydrogen-ion concentration at which the rate of the reaction may be many times greater than at other hydrogen-ion concentrations. Since enzymes appear to possess many of the properties of emulsoid colloids, this result is to be expected. The most usual type of curve obtained is a symmetrical one.

REVERSIBILITY OF ENZYME REACTIONS

In the case of an equilibrium reaction such as the hydrolysis of an ester, for example the hydrolysis of ethyl acetate catalysed by hydrochloric acid, to ethyl alcohol and acetic acid, the hydrolysis of the ester to acid and alcohol, as well as the reverse reaction of

synthesis of ester is accelerated by the catalyst. Thus, it was shown as far back as 1862, by Berthelot and Péan de Saint-Gilles, that if 1 gram-molecule of ethyl alcohol be mixed with 1 gram-molecule of acetic acid, the reaction will go forward until the final composition of the mixture is:

$$\tfrac{1}{3} \text{ mol. alcohol} + \tfrac{1}{3} \text{ mol. acid} + \tfrac{2}{3} \text{ mol. ester} + \tfrac{2}{3} \text{ water.}$$

Exactly the same end-point is reached if the reaction is commenced with 1 gram-molecule of ethyl acetate and 1 gram-molecule of water. In any mixture of these four substances, two opposite reactions are going forward at unequal rates until a certain relative concentration of the components results; when this point is attained, the two reactions have the same velocity. Thus the formation of alcohol and acid, or ester and water, is accelerated by the catalyst. If enzymes are catalysts, and if the reaction they accelerate be a reversible one, the same enzyme which brings about hydrolysis will also accelerate the synthetic reaction. As J. J. Thomson and Nerst have pointed out all reactions are reversible theoretically, and in living organisms there are a large number of reversible or balanced reactions taking place in the cell. Starch, for example, is stored, during the hours of photosynthesis, as a temporary reserve, and later is hydrolysed to soluble sugars. The starch is synthesized from sugars, and, under certain conditions, is hydrolysed back to these once more.

Croft Hill* was the first to demonstrate that a hydrolytic enzyme is also capable of accelerating the synthetic side of the reaction. He allowed maltase prepared from yeast to act upon a strong solution of glucose, when he obtained maltose in the reaction mixture. Actually, both maltose and another disaccharide, iso-maltose, was found. This iso-maltose is an optical isomer of maltose and is a β-glucoside. Iso-maltose is not hydrolysed by maltase, but is hydrolysed by emulsin. If the contention originally suggested by Van't Hoff be correct, namely that an enzyme only synthesizes the same body as it hydrolyses, the presence of iso-maltose in this reaction must have been due to action of some other enzyme than maltase. Croft Hill's enzyme solutions were prepared from ordinary brewer's yeast, and could not have been pure for maltase, and probably contained emulsin as well.

H. E. Armstrong and Gosney† have investigated the system;

* *Trans. Chem. Soc.*, 1898, **73**, 634.

† *Proc. Roy. Soc.*, 1914, **88B**, 176.

oleic acid-glycerol fat-water under the action of lipase. Fig. 22 shows the series of curves obtained. It should be noticed that (1) the greater the concentration of water, the nearer is the equilibrium position to that of complete hydrolysis (upper three curves); and (2) the presence of an excess of glycerol leads to increase of synthesis by the removal of water as well as by mass action.

In a number of cases, the synthetic activity of an enzyme has not been proved. Invertase is a case in point, but, as Bayliss has

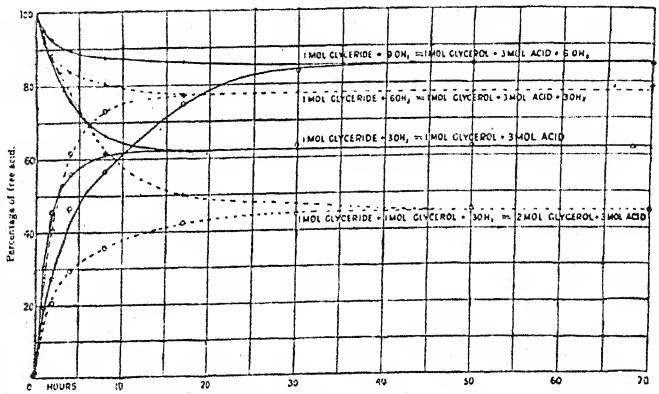


FIG. 22.—Curves showing the different equilibrium positions of an oleic acid-glycerol-fat-water system obtained by the influence of lipase in the presence of different concentrations of water. (After H. E. Armstrong and Gosney. From Bayliss, *Principles of General Physiology.*)

pointed out, even a small amount of synthesis may be of great importance. When starch is acted upon by amylase, the hydrolysis proceeds almost to completion; but if it be assumed that even 1 per cent of starch is synthesized when the amylase acts upon either maltose or dextrin, since the starch that is synthesized in this way is an insoluble compound, equilibrium can have only a momentary existence, so that more starch will be formed to replace that thrown out of solution. The storage of starch in the plant cell may possibly be brought about in this way.

CO-ENZYMES AND ANTI-ENZYMES

It was found by Bertrand that the addition of small amounts of manganese salts considerably increased the oxidizing power of

lactase, and he coined the term *co-enzyme* to express this accelerating power of manganese salts. The term co-enzyme is now used in a rather different sense. It has been found, for example, that when liver lipase is dialysed it gradually looses its power of hydrolysing fats. When, however, the dialysate is added again, the hydrolytic power is recovered. Evidently liver lipase is composed of more than one component, and each separately is inactive. In the case of liver lipase, this dialysable component of the system is thermostable, for the addition of boiled dialysate will reactivate the enzyme. The non-dialysable portion loses its activity when heated, and this may be looked upon as the enzyme proper, while the thermostable, dialysable fraction may be termed the co-enzyme.

Products are known which are antagonistic in their activities to enzyme action; these are termed *anti-enzymes*. Little is known about their properties except their antagonistic action. It is probable that they themselves are not enzymes, since they are able to tolerate temperatures which destroy enzymes. Intestinal worms furnish a good example of anti-enzyme activity. These parasites are able to survive proteolytic enzyme activity in the intestine for the whole of their life period. If intestinal worms be ground up with sand and the mixture be submitted to high pressure, the expressed juice, upon filtering and addition of alcohol, gives a precipitate. This precipitate, after washing with alcohol and ether, and drying over sulphuric acid *in vacuo*, yields a sticky solid, which is soluble in water. When added to either a pepsin or trypsin solution, it will stop their hydrolytic activity.

Zymogens

Since enzymes are formed by the agency of protoplasm, there must be a stage or stages in the course of their formation when they possess no catalytic activities. These precursors of enzymes can in certain cases be isolated from the cell and converted by different methods into the enzyme, when they are called *zymogens*. In the pancreatic juice, trypsin is secreted as the inactive zymogen and enters the duodenum in this condition. When it has entered the duodenum, it comes into contact with another enzyme, enterokinase, which converts it into active trypsin. Similarly, pepsinogen, the zymogen of pepsin, has no action on meat proteins, and can be isolated from the cells of

the stomach wall, and activated by treatment with very dilute hydrochloric acid.

SPECIFICITY OF ENZYMES

The problem of specificity of enzymes is a complex one and has been discussed in detail by Haldane (*Enzymes*, Chapter VI, p. 93). The question arises as to how specificity should be defined. For example, invertase hydrolyses cane sugar and not maltose, and maltase hydrolyses maltose and not cane sugar, while lactase will hydrolyse neither cane sugar nor maltose, but hydrolyses milk sugar. These enzymes are highly specific with regard to the substrate which they will attack. On the other hand, there is the case of yeast zymase and muscle zymase which act upon the same substrate, glucose, the former giving rise to ethyl alcohol and carbon dioxide, and the latter yielding lactic acid.

The enzymes concerned in the hydrolysis of disaccharides and glucosides are highly specific in nature. In the case of the methyl glucosides, four isomerides are known, α-methyl-*l*-glucoside-β-methyl-*l*-glucoside, α-methyl-*d*-glucoside, β-methyl-*d*-glucoside. Neither α-methyl-*l*-glucoside, nor β-methyl-*l*-glucoside are attacked by enzymes, but α-methyl-*d*-glucoside is hydrolysed by maltase, and β-methyl-*d*-glucoside by emulsin, and emulsin is unable to hydrolyse the α-glucoside and maltase unable to hydrolyse the β-glucoside.

We have already considered the case of cane sugar, maltose and lactose. In chemical constitution, these disaccharides only differ in the arrangement of various groups about a central carbon skeleton, but, in relation to enzymic hydrolysis, they exhibit a high degree of specificity. This specificity led E. Fischer to put forward his "lock and key" simile. The enzyme was supposed to have a structure which fits a particular disaccharide as the grooves of a key fit the wards of a lock. Bayliss, however, has suggested the analogy of "master keys," which can open several different locks as being more appropriate in this connection.

The lipases or fat-splitting enzymes do not show the same high degree of preferential specificity as the hexasidases. They do, however, exhibit a quantitative specificity of another kind, inasmuch as they are able to attack optical antipodes at different rates. It was found by Dakin* that in a mixture of the menthyl esters of *d*- and *l*-mandelic acid, pancreatic lipase hydrolyses

* *J. Physiol.*, 1904, **30**, 253.

menthyl-*d*-mandelate more rapidly than menthyl-*l*-mandelate, so that the mandelic acid obtained is strongly dextrorotatory.

The specificity of the proteolytic enzymes furnishes an extremely complex problem, and the reader must be referred to Haldane (loc. cit.) for an adequate discussion of the subject.

CHAPTER VIII

PHOTOSYNTHESIS

THE green plant is able to manufacture carbohydrates from water and the carbon dioxide of the air, and at the same time evolves oxygen. This process can only take place in the presence of light, and in cells containing the green colouring matter chlorophyll. The ability of green plants to synthesize carbohydrates from the two simple initial products, carbon dioxide and water, is of extreme importance, for upon it depends practically the whole of the life of this planet.

This process has been variously termed photosynthesis, photosyntax, carbon assimilation and carbon dioxide assimilation. Somewhat academic objections have been raised to the use of any of these terms. The usual terms used in this country are photosynthesis and carbon assimilation.

How green plants obtain their supplies of carbon had been a mystery for many years, and the first investigator to discover any clue to the problem was the English divine, Joseph Priestley. In 1771 Priestley found that sprigs of mint were able to produce "dephlogisticated air," i.e. oxygen, from an atmosphere vitiated by animal respiration. The Swedish chemist, Scheele, however, was unable to repeat Priestley's results and denied that plants have this power. Priestley's further repetition of his own experiments gave very contradictory results. He entered into a long controversy with Ingen-Housz on the subject, and unfortunately his scientific investigations were brought to an abrupt conclusion, for his theological views brought him into angry conflict with the citizens of Birmingham; his roof was burnt over his head, and he and his family fled to America.

Both Priestley and Scheele had overlooked the important factor of light and did not realize that the process ceased in the dark. It remained for Ingen-Housz (1779) to correlate the discrepancies between Priestley and Scheele, and to show that green plants, like animals, evolve carbon dioxide of respiration in the dark. Further, he was able to show that it is only the green parts of plants that are able to purify the vitiated air. Ingen-Housz's results and his interpretation of them appears to have angered Priestley and hurt his pride. The two carried

out a considerable controversy on the matter, which was prejudiced and bitter on Priestley's side. The latter even went so far as to query some of Ingen-Housz's results and to take others as being his own. The Geneva priest, Senebier, also entered into the controversy, and claimed practically the whole of Ingen-Housz's work as his own.

The next important advance made in the history of the subject was by de Saussure (1804), who introduced quantitative methods of investigation. His experimental methods were admittedly crude, and although it is usually considered that his results show that the volume of oxygen given out is equal to the volume of carbon dioxide absorbed in assimilation, his own figures do not indicate this result; although, if due allowance be made for the crudeness of the technique employed, this is quite a legitimate conclusion. Two other important observations were made by de Saussure. In the first place, he showed that in the sunflower, absorption of carbon dioxide is followed by an increase in dry-weight, and secondly, that plants growing in water or sand increased their carbon content, although their sole source of carbon supply was carbon dioxide.

It is an odd fact that these fundamental discoveries of Priestley, Ingen-Housz and de Saussure do not appear to have been realized by the botanists of that time. They were probably too concerned with the problems of taxonomy, and too busily occupied in classifying plants on the Linnean system to grasp the importance of the discoveries that had been made. At any rate these enormously important discoveries apparently went unheeded, and the belief that plants obtained their carbon supplies from the humus of the soil was accepted. It was largely due to the activity of the German chemist Liebig (1840) that it came to be realized that the sole source of carbon in the green plant is the carbon dioxide of the air, and the humus theory was ultimately abandoned.

Although von Mohl and Unger considered that the first products formed in the leaf were carbohydrates, it remained for Sachs to produce definite proof. By means of the well-known iodine test for starch, he was able to prove that starch is formed in the leaf after exposure to light and that it disappears during darkness, or as Sachs put it: "Starch is the first visible product of assimilation." This generalization is only true to a point; many monocotyledons do not produce starch as a result of photosyn-

thesis, and among the dicotyledons the Umbelliferae, Gentianaceae, and some of the Compositae form no starch as a result of assimilation.

Photosynthesis may be summarized by the equation:

$$6CO_2 + 6H_2O = C_6H_{12}O_6 + 6O_2$$

on the assumption that a hexose is the first sugar formed in the process.

QUALITATIVE DEMONSTRATION OF PHOTOSYNTHESIS

The evolution of oxygen in photosynthesis is readily demonstrated by the use of such aquatics as Elodea or Potamogeton. Sprigs of Elodea placed in water and exposed to sunlight, evolve a stream of bubbles from the cut ends. The gas can be collected by inverting a funnel over the shoots and an inverted test-tube filled with water over the funnel. It will be found that a glowing splint placed in the test-tube will burst into flame. The Engelmann bacterial method may also be used to demonstrate the production of oxygen in photosynthesis. A thread of Spirogyra is mounted in water containing bacteria, such as *Pseudomonas fluorescens*, covered with a cover glass, which is sealed to prevent entrance of air. On exposure to light the bacteria will be seen to swarm round the assimilating filament, owing to the presence of oxygen, and when the preparation is darkened, the movement of the bacteria gradually ceases. Indigo-carmine, or nigrosin which has been reduced to the leuco-compound with sodium hydrogen hyposulphite ($NaHSO_2$), is yet another method of demonstrating the evolution of oxygen during photosynthesis. If a sprig of Elodea be placed in a solution of the reduced dye contained in a closed vessel, and the whole exposed to light, the reappearance of the dye will be observed around the green leaves owing to oxidation of the leuco-base by the oxygen evolved in photosynthesis.

The iodine test may be used to demonstrate the production of starch as a result of assimilation in those leaves that do form this product. The demonstration of carbohydrates as a result of photosynthesis in leaves which do not form starch, is more difficult. To show the production of starch in leaves as a result of assimilation, the plant is placed in the dark overnight and a few leaves removed and killed in steam and the chlorophyll removed with warm alcohol. The leaves are then immersed in a solution of iodine in potassium iodide. Any starch present

will be stained a deep blue by the iodine. If the exposure to darkness has been sufficiently prolonged, little or no starch will be found to be present. The plant is next exposed to light, and after some hours, the leaves are again tested for starch, when it will be found to be abundantly present.

QUANTITATIVE METHODS OF ESTIMATING PHOTOSYNTHESIS

The various quantitative methods that have been devised to measure the rate of photosynthesis may be divided into three groups. (1) Measurement of the amount of oxygen evolved, (2) measurement of the carbon dioxide absorbed by the assimilating organ and (3) estimation of the amount of photosynthate formed.

Measurement of Oxygen Evolved. (Bubble-counting Technique.)—In this method the number of bubbles given off by water plants in unit time is counted. The method suffers from the disadvantage that the size of the bubbles may vary with variations in the surface tension of the water, and further, that they may not be composed of pure oxygen, since a certain amount of nitrogen will almost certainly be present. Numerous attempts have been made to improve the method. The best device is that of Wilmott.* The end of a cut shoot of an aquatic, e.g. Elodea, is placed in a small glass cap or "bubbler," inserted in a glass tube open at one end (Fig. 23) which is filled with distilled water. On illumination the bubbles of gas pass out of the end of the nozzle of the cap into the distilled water in the tube. The size of the bubbles will be constant, since the nozzle is of fixed size and changes in surface tension are prevented by the use of distilled water in the tube, the surface tension of which will remain constant. The presence of nitrogen in the bubbles, as well as the initial error due to the diffusion of oxygen through the water containing the assimilating material, is removed by charging the water with oxygen by vigorous shaking for a long time with pure oxygen from a cylinder.

Warburg† has used a modification of the Haldane-Barcroft apparatus for estimating the rate of photo-synthesis of the unicellular algar, Chlorella. The use of this type of apparatus involves the assumption that the ratio $O_2/CO_2 = 1$. For the experimental details of this apparatus, the original paper should be consulted.

* *Proc. Roy. Soc. (Lond.)*, 1921, 92B, 304. † *Biochem. Zeit.*, 1919, **100**, 230.

The metal palladium has also been used for estimating the amount of oxygen evolved in photosynthesis by land plants. Initially, the leaf, which is contained in a small closed chamber, is surrounded with an atmosphere of hydrogen and carbon dioxide. After a period of illumination, the gas in the chamber is circulated over palladium black. The whole of the oxygen evolved combines with some of the hydrogen to form water. As a result there is a reduction in the volume of gas, which is measured

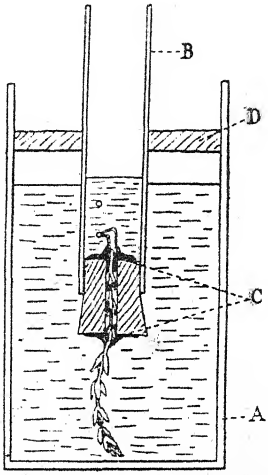


FIG. 23.—Wilmott Bubbler. A is a flat-sided glass jar in which is placed the experimental solution. B is the "bubbling cup" which contains distilled water. Into the base of B is fitted a cork and the "bubbler" and plant (C). A wax mixture is placed round the top and base of the cork to hold the bubbler and plant in position and also to separate completely the liquids in A and B. (After Wilmott.)

by means of a gas burette, and the oxygen output can be calculated.

Measurement of Carbon Dioxide Absorbed by Assimilating Organ. (Continuous Current Method.)—Of all the methods of estimating the rate of photosynthesis, the so-called "continuous current" method is probably the most accurate and certainly the most readily controlled. The assimilating material is contained in a special chamber, and a current of air containing a known proportion of carbon dioxide is passed through. After its passage through the chamber, the carbon dioxide in the issuing gas is estimated.

The amount absorbed by the leaf or other assimilating organ

gives the *apparent assimilation*. To obtain the value of the *real assimilation*, the carbon dioxide of respiration must be added to this value. The carbon dioxide of respiration is estimated by conducting similar experiments in the dark under exactly parallel conditions.

It is not possible to give the details of the various continuous current methods here, and a few of the more important devices that have been used will be found in the papers of F. F. Blackman, Matthaei,* F. F. Blackman and Matthaei, Willstätter and Stoll, and Spoehr and McGee.†

Blackman and Matthaei used baryta water and subsequent titration with acid for estimating the carbon dioxide, whereas Willstätter and Stoll absorbed the gas in soda-lime and determined the gain in weight. Spoehr, like Blackman and Matthaei, used baryta water for absorbing the carbon dioxide, but determined the amount by determining the changes in electrical conductivity.

In these experiments with the continuous current method, it is usual to employ cut leaves. The practical difficulties in the way of using whole plants are many; the space occupied, the temperature control and equal illumination of all the leaves complicate the situation. On the other hand, the assimilation rate of cut leaves is certainly different from that of normal leaves attached to the plant. The question of the translocation of the photosynthetic products, for example, is eliminated in cut leaves, and it would therefore be expected that the assimilation rate would be lower than that of normal attached leaves owing to the accumulation of photosynthate. In actual practice, it has been found that the assimilation rate of cut leaves is higher. The reason is unknown. Dastur‡ has further improved the continuous current method so as to allow of the assimilation rate being followed in leaves attached to the plant.

Change in Alkalinity Method.—In this method, which has been much used by Osterhout and A. R. C. Haas,§ the increase in the alkalinity of a weak solution of sodium bicarbonate containing the assimilating plant material is measured. The material mainly used by Osterhout and Haas was the alga Ulva. This was exposed to light in a closed tube containing sea-water to which was added a definite amount of phenolphthalein. The increase in

* *Phil. Trans. Roy. Soc.* (*Lond.*), 1904, **197B**, 47.
† *J. Ind. Eng. Chem.*, 1924, **16**, 128.
‡ *Ann. Bot.*, 1924, **38**, 779.
§ *Proc. Nat. Acad. Sci.*, 1918, **4**, 85.

alkalinity during assimilation was determined by the change in colour of the indicator, which was compared with a series of similar tubes containing different buffer solutions of known pH and the same concentration of indicator as in the tube containing the assimilating material. It was ascertained that over the range of pH investigated, the amount of oxygen evolved is approximately a linear function of the pH change in the medium. Thus the change in the pH can be taken as a measure of the apparent assimilation. To obtain the value of the real assimilation, the respiration of the tissue is followed in the dark, and the proper correction made.

Estimation of Amount of Photosynthate formed in Assimilation (Sach's Half-Leaf Method.)—In this method, at the commencement of an experiment, one-half of a leaf blade in each leaf of a sample is cut and portions of definite area removed. The remaining half of the leaf blade is left attached to the plant and exposed to illumination for the desired experimental period. At the end of this time it is removed and portions of known area cut out. It is necessary to avoid the main veins when cutting out these leaf areas. The cut portions are then killed by exposure to steam and dried at 100° C. to constant weight. The increase in weight of the second portion over the first portion will give the value of the apparent assimilation. The question of translocation and respiration must also be considered in this connection. To allow for these two processes, the experiment is reversed by keeping the plant for an equal period in darkness at the same temperature and determining the average loss in dry-weight. This value is added to that obtained on illumination and is considered to give the real assimilation. The results are expressed as grammes increase in dry-weight per unit area of leaf-surface.

The objections to this method are many. Horace Brown and Escombe* have compared the values obtained by the half-leaf method with those obtained by the continuous current method and found marked differences.

It will be observed that there are large discrepancies between the two methods, and it is difficult to account for them. It was suggested by Brown and Escombe that the high values given by the half-leaf method may be partly accounted for by the retention of water at 100° C. by the colloidal elements of the leaf cells. When, however, the very wide differences that were recorded

* *Proc. Roy. Soc. (Lond.)*, 1905, **76B**, 112.

by them for the two methods are taken into consideration, the suggestion scarcely appears to be valid.

Thoday* has made an elaborate reinvestigation of Sach's original method and has pointed out several errors. The first is the so-called "asymmetric error," i.e. the two halves of the leaf might not be the same size. The second possible error is the "shrinkage error." During the day the plant may become pressed for water and this would result in an actual shrinkage in area of the leaves. In *Helianthus annuus*, the shrinkage in area was

Assimilation of Catalpa bignonioides

(*Results Calculated per sq. dm. of Leaf Surface*)

	Dry-Weight Method	*Carbon Dioxide Absorbed*	*Carbohydrates Calculated*
(1)	9·83	1·41	1·76
(2)	7·14	1·43	1·79
(3)	2·60	2·35	2·94
(4)	7·22	2·33	2·92

found to be as great as 5 per cent under conditions favourable to a high rate of transpiration.

Thoday was able to improve the dry-weight method considerably by the introduction of certain modifications. Instead of removing one-half of the lamina, he recommended the use of a special rubber stamper of given area. The leaf blade is then stamped out with a number of squares in indelible ink on either side of the midrib. Equal numbers of these squares are removed in the morning and then at the close of the experimental period. The squares cut out from the leaf in this way are killed in steam and dried to constant weight at 100° C. By means of this simple expedient, Thoday was able to remove both the asymmetric error and the shrinkage error.

PATH OF ENTRY OF CARBON DIOXIDE INTO ASSIMILATORY ORGANS

In the higher plants carbon dioxide enters the assimilatory organs through the stomata; in the algae, as well as other aquatics, such as *Elodea canadensis*, by diffusion through the wet cell walls. In

* *Proc. Roy. Soc.* (*Lond.*), 1909, 82B, 1.

land plants, once the carbon dioxide has entered via the stomata into the intercellular spaces of the leaf, it will pass into the assimilating cells in aqueous solution through the wet cell walls.

The problem of stomatal entry of the carbon dioxide into the leaf was only settled after considerable controversy. The earlier investigators, Garreau and others (1850), considered that carbon dioxide entered through the stomata. This view was challenged by Boussingault (1868), who brought forward evidence that entry was effected through the cuticle. Boussingault blocked the stomata of leaves of *Nerium Oleander* by covering the lower surface with lard, and then placed them together with untreated leaves, which served as a control, in atmospheres containing 30 per cent of carbon dioxide. He found that the leaves in which the stomata were blocked absorbed 70 per cent more carbon dioxide than the untreated leaves, and naturally concluded that the main path of entry was through the cuticle.

It was shown later, however, by F. F. Blackman that the high concentration of carbon dioxide used by Boussingault had a narcotic effect on the untreated leaves. Blackman was able to show by careful quantitative experiments that the main path of entry of carbon dioxide into the leaf from the outside air is through the stomata and not through the cuticle; but if the concentration of carbon dioxide be high, then it exerts a toxic action upon the leaf. Some figures obtained by Blackman for *Nerium Oleander* are given below:

Mean Concentration of CO_2 in per cent	*Absorption of CO_2 in c.c. per Unit Area*		*Ratio of CO_2 absorbed by Vaselined Leaf to that absorbed by Normal*
	Normal Leaf	*Vaselined Leaf*	
6·0	0·07	0·01	0·14
6·3	0·055	0·01	0·20
7·5	0·046	0·017	0·21
14·0	0·180	0·040	0·37
55·0	0·049	0·067	1·30
97·0	0·033	0·060	1·80

Brown and Escombe* were able to confirm Blackman's results and showed that if a leaf is hypostomatous, i.e. stomata are mainly present on the lower surface of the leaf, the respiratory and

* *Proc. Roy. Soc.* (*Lond.*), 1905, **76B**, 112.

assimilatory exchanges of gases take place only through the lower side of the leaf. In hyperstomatous leaves, i.e. stomata mainly present on the upper leaf surface, gaseous exchange only takes place through the upper side. When the leaf is amphistomatous, i.e. the leaf is furnished with stomata on both surfaces, respiratory and assimilatory gaseous exchanges are carried on by both surfaces of the leaf.

In the last-mentioned case, an approximate quantitative relationship was found between the distribution of the stomata on the two surfaces of leaf. When assimilation is taking place in bright sunlight, there is a greater intake of carbon dioxide by the upper leaf surface than would be expected from a mere consideration of the ratio of the distribution of stomata on the two sides of the leaf.

The question of diffusion of gases through small apertures has already been discussed in Chapter V and will not be considered further here.

EFFECT OF EXTERNAL AND INTERNAL FACTORS ON THE RATE OF PHOTOSYNTHESIS

The various factors that influence the rate of photosynthesis may be conveniently divided into two classes: (1) External factors and (2) internal factors. The chief external factors are:

(*a*) Light intensity.
(*b*) Carbon dioxide concentration.
(*c*) Temperature.
(*d*) Water supply.

The chief internal factors are:

(*e*) The protoplasmic factor.
(*f*) Chlorophyll content.
(*g*) Accumulation of products of photosynthesis.

The older investigators placed too much reliance on the effect of one factor at a time on the rate of photosynthesis and paid little regard to the influence of others. It remained for F. F. Blackman* to conceive a scheme which took into account all the other factors when the effect of any one particular factor on the photosynthetic rate was being examined. This is known as the *Blackman Theory of Limiting Factors* and is best stated in the author's own words:

* *Ann Bot.*, 1905, **19**, 281.

"When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the slowest factor."

Blackman supposes, for example, that in a leaf sufficiently illuminated to decompose 5 c.c. of carbon dioxide per hour only 1 c.c. of the gas is present. In such circumstances it is obvious that the energy of the light is more than sufficient to decompose all the carbon dioxide. Similarly if the concentration of the carbon dioxide be raised to 2 c.c., the light intensity is still more than sufficient to decompose this quantity of the gas. As in the last case, the factor limiting the rate of the process is the concentration of the carbon dioxide. If now the concentration of carbon dioxide be raised to 5 c.c., then the light energy is only just sufficient to decompose the concentration of gas and no more. If the concentration of carbon dioxide be raised any further, the rate of photosynthesis will not be increased, as the energy of the light is now insufficient to decompose any greater concentration of the dioxide. Light will now be the factor limiting the rate of the process. If the light intensity be increased, then a higher concentration of carbon dioxide will be decomposed, and the photosynthetic rate will be increased until light is once more a limiting factor. Graphically expressed, the curve will show a rise in the rate of assimilation until intensity of light and carbon dioxide concentration are equally balanced, when a straight line will be shown. If a higher intensity of light is used, then the curve will once more ascend until light is again a limiting factor, when a straight line will once more be obtained (Fig. 24A).

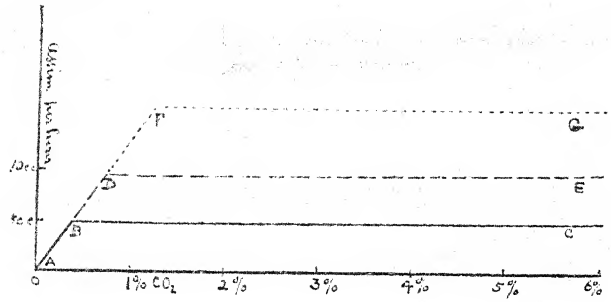
To discover which is the limiting factor, the following principle is applied: "When the magnitude of a function is limited by one set of possible factors, increase of that factor and that factor alone, will be found to bring about an increase of the magnitude of the function."

Matthaei obtained limiting factor curves with *Prunus Laurocerasus* with different intensities of light and variations in temperature from − 10° C. to 30° C. Wilmott,* using his bubble-counting technique, also considered that he had obtained typical "limiting factor" curves with Elodea.

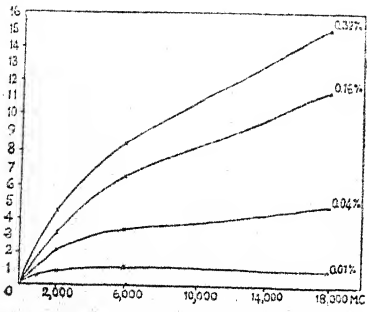
Blackman and A. M. Smith,† using Elodea, determined the rate of assimilation at two different light intensities (5·7 and 8·1 units) and at constant temperature (19° C.), and claimed

* *Proc. Roy. Soc. (Lond.)*, 1921, 92B, 304.

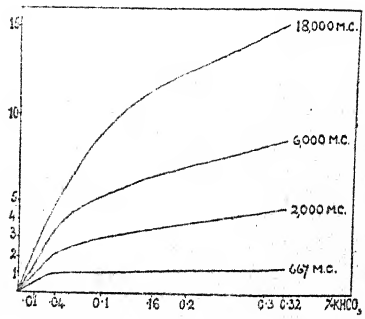
† Ibid., 1911, 83B, 389.



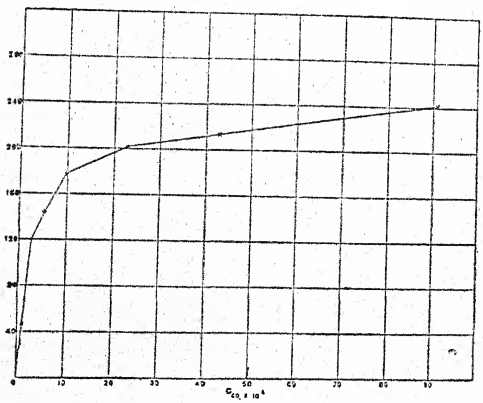
A



B



C



D

FIG. 24.—A. Theoretical "limiting factor" curves for photosynthesis. (After F. F. Blackman.) B. Rate of photosynthesis of Fontinalis in different concentrations of potassium bicarbonate with light intensity of a constant value. C. Photosynthesis of Fontinalis at different light intensities in solutions of potassium bicarbonate of constant concentration. (B and C after Harder.) D. The rate of photosynthesis of Chlorella in different concentrations of carbon dioxide. The ordinate shows the rate of photosynthesis, the abscissa, the concentration of carbon dioxide. (After Warburg). (B, C, and D from Spoehr, *Photosynthesis*.)

to have obtained typical limiting factor curves showing a sharp break when light became the limiting factor.

The theory of limiting factors has been actively attacked during the last twenty years. Hooker* and later W. H. Brown† both submitted the theory to a good deal of destructive criticism. Brown, for example, considered that the curve obtained by Blackman and Smith in their work with Elodea (see above) is in reality made up of two separate curves, and that no sharp break exists in the curve, and therefore there is no operation of limiting factors in the Blackman sense. The whole question here turns on the fact as to whether there is or is not a sharp break in the curve or whether the curve is approximately logarithmic in shape.

Boysen Jensen,‡ using *Sinapis alba* and other plants, and Lundegårdh,§ found a smooth curve to exist and no sharp break occurred when one of the factors became limiting. Warburg,‖ using the unicellular alga Chlorella, also obtained smooth curves. It should be mentioned that these investigators did on occasion obtain a sharp break in their curves. Harder,¶ who used a number of different aquatics, such as the water moss *Fontinalis antipyretica*, *Cinclidotus aquaticus* and two species of Cladophora, has carried out a very thorough investigation of the theory of limiting factors. Solutions of potassium bicarbonate were used as a source of carbon dioxide supply, and the assimilation rates were determined by estimating the amounts of oxygen evolved by a volumetric method. In all the cases investigated, the curves showed no sharp break and were smooth in shape.

Harder tested the matter in two ways: in the first case, all the factors except one were kept constant, and in the second case, variations in two factors were followed. The curves obtained are shown in Figs. 24B and 24C. An important point in Harder's investigations is the fact that the same material was used throughout for each experiment, whereas Blackman and his colleagues used fresh samples of material for each experiment, and the points of their curves lie on such an irregular line that it is difficult to say whether they are either continuous or show a sharp break. An examination of Harder's second series of determinations in which two factors were varied together show that an increase in

* *Science*, 1917, **46**, 197.
† *Phillipine J. Sci. C. Bot.*, 1918, **13**, 345.
‡ *Bot. Tidssk.*, 1918, **36**, 219.
§ *Svensk. Bot. Tidssk.*, 1921, **15**, 46.
‖ *Biochem. Zeit.*, 1919, **100**, 230.
¶ *Jahrb. f. wiss. Bot.*, 1921, **60**, 531; *Ber. deut bot. Ges.*, 1923, **41**, 194.

either factor brings about an increase in the rate of assimilation. According to Harder, the factor which is most important is the factor which is in relative minimum. In other words, the evidence at present available relating the interaction of factors of light intensity and carbon dioxide concentration, appears to be that a factor is only "limiting" in the Blackman sense when it is very weak, or to use Harder's own expression, "in minimum."

James* has been able to confirm the results obtained by Harder and Warburg. The material used was *Fontinalis antipyretica.* When a low light intensity was employed (20 arbitrary units) and at a temperature of 19° C. and low concentrations of carbon dioxide, the curves obtained took the form of an oblique hyperbola. When higher concentrations of carbon dioxide were used and the same light intensity, the rate of assimilation was found to be independent of the concentration of carbon dioxide over the range 3 to 5 per cent. If the two factors of light intensity and carbon dioxide concentration were varied simultaneously, similar curves to those of Harder were obtained.

The theory of limiting factors applied to land plants is by no means so simple as in the case of aquatics. In an important investigation, Maskell† has shown that variations in the uptake of carbon dioxide by the leaf are due to variations in such matters as the variations in stomatal resistance and the remaining resistances of the leaf. The stomatal resistance, for example, is dependent on the season of the year. The range of stomatal opening was found to vary with the different months of the year under conditions of constant light intensity. A well-marked diurnal rhythm in stomatal opening was also discovered with the plant used in this investigation, *Prunus Laurocerasus* var. *rotundifolia.* The previous history of the leaf and the previous moisture relations of the leaf must also be taken into account.

The matter is fully developed by Maskell from the mathematical standpoint and the original paper should be consulted for full details. The present position of the theory of limiting factors is well summarized by Maskell. As he has pointed out, the theory was originally put forward as a first approximation and has been misinterpreted as a rigid law. If it be regarded as a clue to the interpretation of phenomena "the general principle of limiting factors as suggested by Blackman in 1905 cannot yet be replaced."

* *Proc. Roy. Soc.* (*Lond.*), 1928, **103B**, 1.

† Ibid., 1928, **102B**, 467, 488.

EXTERNAL FACTORS

Influence of Carbon Dioxide Concentration on Assimilation.—It was generally recognized by the older investigators that an increase in the concentration of carbon dioxide brought about an increase in the rate of assimilation. Warburg,* working with Chlorella, at a temperature of 25° C., found that with low concentrations of carbon dioxide, the rate of photosynthesis is directly proportional to the concentration. When the concentration of carbon dioxide is progressively increased, there is a continuously smaller increase in the rate of assimilation until it appears to be quite independent of the concentration. Warburg places the following interpretation upon his results: (*a*) that the rate of assimilation is proportional to the concentration of carbon dioxide and (*b*) is proportional to some second unknown substance which reacts with the carbon dioxide (see below).

It was ascertained by James,† in his investigations with *Fontinalis antipyretica*, that when he used sodium bicarbonate as a source of carbon dioxide in place of carbon dioxide in solution, with the same rate of flow per hour (400 c.c.), the sodium bicarbonate gave rise to a higher assimilation rate than a pure solution of carbon dioxide of equal partial pressure when no other factor was limiting. When the rate of flow was increased to 600 c.c. per hour with low light intensity and consequently of slow assimilation, the two solutions gave the same rate of assimilation. It would appear that, in solutions of sodium bicarbonate, it is only the free carbon dioxide that is available for photosynthesis. When the light intensity was increased from 20 to 80 units, the bicarbonate solutions gave a greater assimilation rate than pure solution of carbon dioxide of equal partial pressure, although there was no increase in the rate of assimilation with increase of flow of bicarbonate solution with this intensity of light as there was when the light intensity was low. James considers that the assimilation rate appears to be proportional to the concentration of carbon dioxide at low concentrations, as has been shown by previous investigators, but since this factor disappears with a faster flow of liquid, it is probable that the linearity of the curves obtained was due to the conditions of diffusion obtaining in their experiments rather than to internal stages in the photosynthetic process.

* *Biochem. Zeit.*, 1919, **100**, 230. † *Proc. Roy. Soc.* (*Lond.*), 1928, **103**B, 1.

F. T. McLean,* working in the Philippine Islands under field conditions, found that the rate of assimilation of coconut leaves reached a maximum in the morning, and this was followed by a depression at midday, and a second increase occurred in the afternoon. When cut leaves were used, only a single maximum was discovered. It is difficult to account for this last result. It is possible that the limiting factor in operation was the accumulation of the products of photosynthesis, as the normal channel of translocation had been removed.

In general terms, a high concentration of carbon dioxide exerts a narcotic effect upon the assimilation rate. In an atmosphere of pure carbon dioxide, photosynthesis comes to a standstill. The concentration of carbon dioxide in the normal atmosphere is small (about 3 parts in 10,000) and numerous attempts have been made from time to time to determine whether an increase in the concentration of the gas will bring about an increase in crop yield. As long ago as 1885, Kreusler attempted this problem, and the results that have been obtained since that time have been very contradictory.

A more recent investigation has been carried out by Bolas and Henderson.† The carbon dioxide used for this work was especially purified, and working under controlled conditions these investigators discovered that artificial enrichment of the atmosphere with carbon dioxide brought about an increase in dry-weight of cucumber. The increase became evident at an early stage of growth, usually from two to three days from the beginning of an experiment. In one particular instance, the carbon dioxide concentration was increased to 31·3 parts in 10,000, when it was found that the plants showed a percentage increase in dry-weight of $60 \cdot 6 \pm 8 \cdot 5$ over the controls with 3·9 parts of the gas in 10,000. The very contradictory results obtained in the older investigations were probably due to the fact that the gas employed was very impure. For example, carbon dioxide from blast furnaces and gas flues was used. The carbon dioxide from such sources is always mixed with considerable amounts of sulphur dioxide and hydrogen sulphide which are highly toxic to plants.

Light Intensity.—The older work on this subject appeared to indicate that, with low light intensities, the rate of photosynthesis was proportional to the light intensity, while, with higher intensities of light, some other limiting factor came into play. Much

* *Ann. Bot.*, 1920, **34**, 367.

† Ibid., 1928, **24**, 509.

reliance cannot be placed on these investigations owing to the fact that the experimental technique employed was far from satisfactory.

F. F. Blackman and Matthaei,* using the continuous current method of estimating photosynthesis and ordinary sunlight as a source of illumination, have made a number of important observations in this connection. With *Helianthus tuberosus* and at a temperature of 18·0° C. to 18·3° C. and a carbon dioxide concentration of 2·5 per cent on a day in July, they obtained the following results:

July 30, 1904

Time (p.m.)	*Conditions of Illumination*	*Temperature*	*Real Assimilation in Square Decimetres per Hour*
12.30–1.30	—	—	Preliminary
1.30–2.30	Heavy clouds	18·2	0·0030
2.30–3.30	Violent thunder storm, clearing up later	18·3	0·0060
3.30–4.30	Brighter, no rain	18·3	0·0118
4.30–5.30	Sun at first. Then clouded over, storm driving up	18·3	0·0086
5.30–6.30	Overcast. Steady rain. Heavy storm at 6.10	18·0	0·0020

It will be seen from the values given in the table that the rate of assimilation varied markedly with the intensity of the light. According to Blackman, equal intensities of light incident on equal leaf areas bring about the same amount of photosynthesis, provided that light is the limiting factor and temperature does not involve the time factor. The amount of light required by a leaf is a specific value for a given temperature. For example, at 29·5° C., *Helianthus tuberosus* can assimilate at twice the rate of *Prunus Laurocerasus*, but it requires twice the amount of illumination: 0·36 full sunlight for *P. Laurocerasus* and 0·69 for *H. tuberosus*.

In certain ecological investigations of sun and shade plants, Lundegårdh† found for *Oxalis Acetosella* (a shade plant) that,

* *Proc. Roy. Soc.* (*Lond.*), 1905, **76**B, 402.
† *Svensk. Bot. Tidssk.*, 1921, **15**, 46.

with a carbon dioxide concentration of 0·57 mg. per litre, the assimilation rate was increased in direct proportion to light intensity up to 0·05 to 0·1 of direct sunlight. If the light intensity were increased beyond the higher limit, there was no further increase in the rate of photosynthesis. Increase of the concentration of carbon dioxide, even with such low intensity of light as 0·025 of direct sunlight, brought about an increased rate of assimilation. On the other hand, with sun plants, such as *Nasturtium palustre* and *Atriplex latifolia*, a different relation was found to exist between rate of assimilation and intensity of light. With low intensity of light, the photosynthetic rate was directly proportional to light intensity, but when this latter factor was increased the curve became logarithmic, whereas with shade plants, typical Blackman "limiting" factor curves were obtained. Lundegårdh has suggested from his results that there is a considerable ecological significance to be found in these facts. The habitat of shade plants usually have a carbon dioxide concentration approximately double that of the normal atmosphere; so that, in spite of low light intensity, plants inhabiting such situations are able to synthesize relatively large amounts of carbohydrates.

Intermittent Light.—Warburg* has conducted experiments on the action of intermittent light on photosynthesis. By means of a special rotatory disc perforated with sectors he was able to expose the unicellular alga, Chlorella, to equal periods of illumination, not of equal experimental times. With high intensities of light, the rate of photosynthesis was greater in intermittent light than in continuous light of the same intensity, and further, the more rapid the alternation of light and dark, the greater was the rate of photosynthesis. There are two possible explanations to account for this result. Either photosynthesis may have continued at an undiminished rate in darkness, or alternatively, the assimilation rate was lowered in darkness and doubled in the light. Warburg considers this second explanation the more probable. During the period of darkness, carbon dioxide will still be entering the cell, and synthesized products will be diffusing away from the active centres of synthesis, and both these factors would tend to increase the rate of photosynthesis.

Effect of Light of Different Wave-Lengths.—The older work on this aspect of photosynthesis is largely worthless. The earlier

* *Biochem. Zeit.*, 1919, **100**, 230.

investigators failed to realize that unless the light of different wave-lengths possessed the same energy value, the data obtained could not be compared. Actually the fundamental question to be decided is what is the relationship between light of different wave-lengths and the proportion of absorbed energy used by the plant in assimilation. Therefore, not only must the energy value of the light be the same, but the amount of light absorbed by the leaf must also be the same.

Engelmann, using filaments of green algae and his bacterial technique (see above), concluded that two maxima occur, one in the red and the other in the blue end of the spectrum. Kniep and Minder,* working with Elodea, realized that the energy values of the different wave-lengths of light must be the same, but unfortunately they employed the old bubble-counting method of measuring assimilation, so that little reliance can be placed upon their results. They concluded from a large number of determinations that with light of the same intensity, red and blue light bring about the same degree of photosynthesis, but there is no assimilation in green light. Timiriazeff passed light through a spectrum on to a leaf and observed the amount of starch formed in different regions of the spectrum. The experimental method employed is not very satisfactory. It does not follow that the rate of formation of starch is identical with the rate of assimilation. Moreover, it has been pointed out by Snag that the formation of starch might well have been due to the action of heat rays. The leaf already contains sugars, and the heat rays might well interfere with the starch-sugar equilibrium of the leaf cells. Leaving these objections aside, however, Timiriazeff found that the blue and violet rays exerted very little effect on starch formation, but that there was a significant increase of starch in the red end of the spectrum.

Ursprung† has repeated Timiriazeff's work, using *Phaseolus multiflorus*, and found that starch formation occurs throughout the range of the visible spectrum. There was no starch formation in the infra-red region. In the ultra-violet, starch formation occurred up to 330 $\mu\mu$. The lowest limit of starch formation was found in light of wave-length 759 $\mu\mu$. From these results there would seem to be little doubt that starch formation can occur in all light between 759–330 $\mu\mu$.

* *Zeit. f. Bot.*, 1909, **1**, 619.
† *Ber. deut. bot. Ges.*, 1917, **35**, 44; 1918, **36**, 73.

Wurmser,* using Osterhout and A. R. C. Haas' pH method of estimating the rate of photosynthesis, discovered the following values for *Ulva lactuca* in different wave-lengths of light, and at the same time was able to obtain the ratio between the rate of photosynthesis and the amount of energy absorbed:

Wave-Length in μμ	*Assimilation (x)*	*Energy Absorbed (E)*	$\frac{x}{E}$
750–560 (red)	100	100	1·00
560–460 (green)	24	6	4·00
460– (violet)	80	34	2·35

It can be seen from this table that although only a small amount of green light was absorbed compared with the red, yet the green light was used in assimilation to about four times the extent of the red rays and approximately twice that of the violet rays.

Although these results of Wurmser have been adversely criticized by Warburg and Negelein,† they are nevertheless of considerable value. Warburg and Negelein working with Chlorella found that in red and yellow light more than 97 per cent of the incident radiation was absorbed, while in the blue region more than 99 per cent was absorbed. They found that there was little or no relation between the assimilation rate and the absorption bands of chlorophyll.

Bonnier and Mangin considered that assimilation was possible in the ultra-violet region of the spectrum. Such a possibility, however, is not very probable. The intensity of the ultra-violet rays at low sea-levels is small, and again photosynthesis can take place under conditions in which the presence of ultra-violet rays is excluded. It is also very doubtful if the infra-red rays play any significant part in photosynthesis although chlorophyll can absorb infra-red rays to a certain extent.

Temperature.—According to the Van't Hoff law, the rate of a chemical reaction is doubled or trebled for every 10° C. rise in temperature. The temperature coefficient, Q_{10}, represents the ratio of the rate of a reaction at one particular temperature to

* *Bull. Soc. Chim. biol.*, 1923, **6**, 487; *Ann. Physiol. Physiochim., Biol.*, 1925, **1**, 47.
† *Zeit. physikal. Chem.*, 1922, **102**, 235; 1923, **106**, 191; *Naturwiss.*, 1922, **10**, 647.

the rate at a temperature 10° C. lower. The determination of the temperature coefficient in the case of photosynthesis presents a number of difficulties as several factors enter into the situation and have to be considered. In the first place there is a photochemical reaction involved, and the temperature coefficient of photochemical reactions is about 1·4 or it may be as low as 1·0. Further, the internal temperature of the leaf may be different from that of the external atmosphere, and this will be affected by high intensities of light, and lastly, respiration must also be corrected for, as the rate of this process will also be affected by a rise in temperature.

The first standard investigations on the influence of temperature on the photosynthetic rate were carried out at the Cambridge Botany School by F. F. Blackman and his co-workers. Matthaei,* for example, showed that with *Prunus Laurocerasus* and *Helianthus tuberosus*, with temperatures from − 6° C. to 25° C., the Van't Hoff law was fairly accurately followed and $Q_{10} = 2 \cdot 1$ for *P. Laurocerasus* and 2·5 for *H. tuberosus*, provided that neither light nor carbon dioxide were limiting factors. A weak intensity of light, however, could limit and determine the course of the reaction. In such circumstances, when light is a limiting factor, increase of temperature does not increase the rate of photosynthesis. Above 25° C. there was a continuous fall in the rate of the process, and the higher the temperature the more rapid the fall. The Blackman "time factor" is involved here; the more prolonged the time during which a leaf is exposed to high temperature, the more rapid is the fall in the rate of the process.

Osterhout and Haas,† using *Ulva rigida* and their pH method of determining the rate of photosynthesis, found that raising the temperature from 17° C. to 27° C. gave a value of $Q_{10} = 1 \cdot 81$, a result which falls into line with the Blackman school.

It would thus appear that when neither light nor the concentration of carbon dioxide is limiting the rate of the process, the Van't Hoff rule is followed in the manner characteristic of many chemical reactions. When, however, light intensity is weak, temperature has little influence, which is characteristic of photochemical reactions. With low intensity of light a photochemical reaction determines the rate of assimilation, whereas when light intensity and concentration of carbon dioxide is high, tempera-

* *Phil. Trans. Roy. Soc.* (*Lond.*), 1904, **197B**, 47.
† *J. Gen. Physiol.*, 1919, **1**, 295.

ture influences the rate as though a chemical reaction were involved, it can be argued that some "dark" chemical reaction is involved in photosynthesis. The rate of this "dark" reaction is determined by temperature and limits the whole process. This is sometimes spoken of as the "Blackman reaction."

The range of temperature at which photosynthesis can take place varies widely with different plants. Matthaei ascertained that measurable photosynthesis occurs in *Prunus Laurocerasus* at — 6° C. The claim has been made that certain lichens can assimilate at as low a temperature as — 20° C., while Opuntia is said to be able to function at as high a temperature as 55° C.

Water.—Water is as necessary as carbon dioxide for photosynthesis to take place. In general terms it can be stated that tissues must be turgid for successful photosynthesis. The older work indicated that with loss of turgidity there was a lowering of the assimilation rate.

Thoday,* using his modification of Sach's half-leaf method of estimating photosynthesis, found that there was a marked relationship between the turgidity of the leaf tissues and the rate of photosynthesis. The following values were obtained for *Helianthus annuus*:

	Average Rate of Assimilation in Milligrammes per Hour per Square Decimetre
Turgid	16·1
Moderately turgid	12·5
Limp	8·5
Limp to flaccid	5·3
Flaccid	1·6

Iljin,† using the porometer for determining stomatal aperture, measured the rate of photosynthesis and the water-content of leaves, and obtained similar results to those of Thoday.

The most exact work on this matter has been conducted by Dastur,‡ using his improved continuous-current method for measuring the rate of assimilation. He ascertained that in ageing leaves of Abutilon, and other plants, there was a loss of photosynthetic activity in the mesophyll tissues bordering the margins of the leaf, i.e. in the cells which lie farthest away from the vascular tissues, and this loss in photosynthetic activity

* *Proc. Roy. Soc.* (*Lond.*), 1909, 76B, 112.
† *Jahr. f. wiss. Bot.*, 1922, **61**, 670; *Flora*, 1923, **116**, 360.
‡ *Ann. Bot.*, 1924, **38**, 779; 1925, **39**, 769.

gradually spread to the middle of the leaf. He was further able to show that the rate of assimilation is directly proportional to the water-content of the leaves. On the other hand, leaves with a lower water-content have a greater assimilation rate per unit area than those with a higher water-content. For example, the leaves of *Abutilon Darwini* have a lower water-content than those of *Cineraria stellata*, and the assimilation rate of the former is greater than that of the latter.

INTERNAL FACTORS

Chlorophyll Content.—Willstätter and Stoll, in the course of their elaborate investigations on the chemistry of the chlorophyll pigments (see below), showed that four pigments are present in the chloroplast, namely, chlorophyll *a*, chlorophyll *b*, carotin and xanthophyll. Quantitative methods were also devised to estimate the amount of chlorophyll present in leaves. Using these quantitative methods to determine the amount of chlorophyll content of leaves, they also estimated the rate of photosynthesis at the same time of the leaves of different plants, and of the same plant under different conditions, e.g. etiolated leaves, autumnal and normal leaves. The rate of photosynthesis was determined by the continuous-current method with a high concentration of carbon dioxide (5 per cent) and a high intensity of light (48,000 to 130,000 lux) at a temperature of 25° C. Thus none of these factors was limiting the rate of photosynthesis. The results were expressed in the form of the ratio:

$$\frac{\text{Assimilation of } CO_2 \text{ in gms. per hour}}{\text{Chlorophyll content of leaves in gms.}}$$

This was termed the *Assimilation Number*. Some of the values obtained are given on p. 211.

It should be mentioned here that all very young leaves as well as old leaves, or leaves poor in chlorophyll, were avoided. It will be seen that the assimilation number for some of these different species is approximately constant while others gave different and higher numbers. In *Helianthus annuus*, the assimilation number varied between 10·9 and 16·7. It follows from these results that in mesophytic plants in which the leaves are fully developed, the photosynthetic rate is not proportional to the chlorophyll content. In autumn leaves considerable variations were recorded. Yellow

leaves, for example, were found to have a higher assimilation number than normal green leaves of the same species.

The assimilation of etiolated plants turning green has led to a good deal of controversy, which has been more or less settled by G. E. Briggs.* It was found by Irving,† who used etiolated seedlings of barley and *Vicia Faba*, that the etiolated shoots had no power of photosynthesis, and that photosynthesis only begins when the leaves are fully green, and then develops very quickly. Irving therefore concluded that chlorophyll is not a limiting

Species	*Temperature*	*10 Grammes Fresh-weight of Leaves used*		*Assimilation Gm. CO_2/Hour per 1 Gm. Dry-weight*	*Assimilation Number*
		Dry-weight Gm.	*Chlorophyll Mg.*		
Tilia cordata ..	25° C.	3·19	28·1	0·028	6·6
Sambucus nigra ..	25° C.	2·75	22·2	0·034	6·6
Prunus Laurocerasus	30° C.	3·40	12·2	0·029	8·1
Helianthus annuus	25° C.	1·72	16·5	0·134	14·0
Acer Negundo ..	25° C.	2·22	24·8	0·086	7·7

factor in assimilation during the early stages of development, and that some other factor must at this time control the rate.

Willstätter and Stoll‡ arrived at a very different conclusion on this matter. Working with *Phaseolus vulgaris* and *Zea Mays*, they found that the assimilation rate increased with great regularity with corresponding increase in the chlorophyll content of the leaves.

Briggs has found that the assimilating power of young leaves is dependent upon their age rather than upon their chlorophyll content. By bringing plants from the dark to the light, he found that the assimilation rate depended on the age of the leaves, i.e. on the number of days that had elapsed from sowing and not on the amount of chlorophyll present. Irving's material was only five days old, while that of Willstätter and Stoll was considerably older, often as much as fourteen or fifteen days. In such plants the photosynthetic mechanism was complete, with the exception of the chlorophyll. Briggs therefore suggested that the "photo-

* *Proc. Roy. Soc.* (*Lond.*), 1920, **91B**, 249; 1922, **94B**, 12.
† *Ann. Bot.*, 1910, **24**, 805.
‡ *Ber. deut. chem. Ges.*, 1915, **48**, 1540.

synthetic potentiality" of this unknown factor increases with age, whether the plant remains in light or darkness.

In some further investigations, Briggs has shown that seedlings can be placed in two classes with regard to their photosynthetic activity. In the first group, which includes such plants as Ricinus, *Zea Mays* and Phaseolus, there is a special photosynthetic organ developed, which is different from the storage organ. In the second group, which contains such plants as Helianthus, Acer and Curcubita, in which the cotyledons are the storage organs, and on germination become the first photosynthetic organs, photosynthetic activity is developed at germination, and there is no lag in the development of the photosynthetic activity and the full formation of chlorophyll.

Protoplasmic Factor.—That there is some other internal factor besides chlorophyll concerned in photosynthesis, has been known for a long time. This has been termed the *Protoplasmic Factor.* Practically nothing is known about the nature of this protoplasmic factor. Willstätter and Stoll have advanced the view that it may be enzymic in nature.

OTHER FACTORS CONCERNED IN PHOTOSYNTHESIS

Oxygen.—Assimilation is unable to take place in the absence of oxygen, but only a very low partial pressure of the gas is necessary for the process to go forward. Friedel* found that the oxygen content could be reduced to 2 per cent, or increased to 50 per cent, without any resultant effect on the rate of photosynthesis. The older workers replaced oxygen by hydrogen and carbon dioxide. Willstätter and Stoll consider that the use of hydrogen is not permissible, since it does not normally form a part of the air surrounding plants, and that its use might have an adverse influence on photosynthetic mechanism and possibly disturb the protoplasm.

According to Willstätter and Stoll, who used atmospheres of nitrogen and carbon dioxide, reduction of the oxygen to a partial pressure of 1/100 of an atmosphere does not interfere with the rate of assimilation, but if oxygen be entirely excluded photosynthesis comes to a standstill. Some plants, however, are more resistant in this respect than others. With Pelargonium, for example, no photosynthesis took place after an exposure of two hours to a current of gas free from oxygen. Cyclamen, on the

* *U.S. Dept. Agric.*, 1901, Bull. 28.

other hand, even after fifteen hours' exposure to an atmosphere free from oxygen still gave slight evidence of photosynthesis. Willstätter and Stoll have suggested from these results that the oxygen is removed from the plant in two stages: (*a*) there is first of all replacement of free oxygen by an oxygen-free atmosphere, and (*b*) in the second place there is removal of loosely bound oxygen, through the dissociation of an oxygen compound, when the oxygen tension of the atmosphere becomes less than that of this hypothetical compound. It is only the more resistant plants that are able to withstand this second phase of oxygen removal, and so long as this second phase has not set in, photosynthesis can still take place.

Acids.—It was at one time considered that the presence of acids in low concentration was able to increase the rate of assimilation in aquatics. It has been shown by Wilmott* that in Elodea the increase in the rate of photosynthesis is due to the decomposition of encrusted carbonate on the plant by the acid with consequent evolution of carbon dioxide. If light be a limiting factor in such circumstances, there is no increase in assimilation rate on the addition of acid.

Accumulation of Products of Photosynthesis.—It would be expected that if the products of photosynthesis were allowed to accumulate in the assimilating cells without removal by translocation, then the rate of the process would be slowed up until eventually it came to a standstill. According to the law of mass action, the rate of a chemical reaction falls off as the products accumulate. The initial rate of a reaction will only be maintained so long as the initial concentrations of the reactants are preserved and the products of the reaction removed. There is a further point in this connection, namely, that the soluble sugars formed in photosynthesis are osmotic substances, and any high concentration of these products might lead to dislocation of the photosynthetic mechanism. The conversion of sugar into starch would obviate this difficulty, but a considerable accumulation of starch may also bring photosynthesis to a standstill, as it reduces the effective surface of the chloroplasts. There is a considerable amount of evidence available to show that accumulation of the products of photosynthesis brings the process to a standstill.

The Compensation Point.—The particular light intensity at which

* *Proc. Roy. Soc.* (*Lond.*), 1921, **92B**, 304.

the processes of photosynthesis and respiration just balance one another, i.e. when they are in a state of equilibrium, has been termed by Plaetzer the *compensation point*. It will be clear that gaseous exchange at the compensation point will be zero. As the compensation point is dependent upon the rate of respiration it will vary in different plants.

CHEMISTRY OF THE PHOTOSYNTHETIC PIGMENTS

The painstaking work of Willstätter and his co-workers has largely elucidated the composition and structure of the pigments involved in photosynthesis. The name chlorophyll was apparently first used in 1818 by Pelletier and Caventou for the green substance of leaves, although as far back as 1689 Nehemiah Grew had succeeded in extracting with oil green and yellow colouring matter from leaves so that even at that time there was evidence that a mixture of pigments was present.

In 1864 Stokes had shown that at least four pigments were present in the chloroplast: two green and two yellow pigments. Twett, using columns of inulin or calcium hydroxide, confirmed this observation, but considered that at least five yellow pigments were present.

The investigations of Willstätter and Stoll have shown that the pigments of the chloroplast consist of two green pigments, chlorophyll *a* $C_{55}H_{72}O_5N_4Mg$ and chlorophyll *b* $C_{55}H_{70}O_6N_4Mg$, which give the characteristic green colour to leaves, an orange-red hydrocarbon, carotin, $C_{40}H_{56}$ and a yellow pigment, xanthophyll, $C_{40}H_{56}O_2$. There is a possibility that xanthophyll may be a mixture of closely related isomeric and isomorphic compounds.

In the Phaeophyceae, a further brown pigment was discovered in addition to the other pigments of the chloroplasts. This has been termed fucoxanthin and has the empirical formula $C_{40}H_{54}O_6$. In the Rhodophyceae there is in the cells a red pigment, phycoerythrin, the chemical nature of which is at present unknown. It may be a protein, but the nitrogen content is low, and it does not give the biuret reaction.

Chlorophyll.—The term chlorophyll is used here to cover the reactions of both chlorophyll *a* and *b*, as these substances behave in a very similar way to different reagents.

Chlorophyll *a* is a blue-black microcrystalline body in the solid state, which shows green-blue with a ruby-red fluorescence

in alcoholic solution. It dissolves readily in ethyl alcohol, benzene, pyridine, carbon disulphide and chloroform and is moderately soluble in methyl alcohol, but is only soluble with difficulty in 80 per cent ethyl alcoholic solution, 90 per cent methyl alcoholic solution and light petroleum. Chlorophyll *b* is a microcrystalline green-black product in the solid condition. It is soluble in the same range of organic solvents as chlorophyll *a*, with the exception of light petroleum in which it is quite insoluble, and 90 per cent methyl alcohol in which it is only slightly soluble. In ethyl alcoholic solution it gives a greener colour than chlorophyll *a* with a slight yellow tinge, and the red fluorescence is tinged with brown.

Both chlorophyll *a* and chlorophyll *b* are neutral bodies, which react readily with acids and alkalis. When treated with dilute hydrochloric acid, oxalic acid or even carbonic acid, magnesium is removed from the molecule and replaced by two atoms of hydrogen and a substance known as phaeophytin is obtained. Chlorophyll *a* gives phaeophytin *a*, and chlorophyll *b* phaeophytin *b*.

Phaeophytin on treatment with zinc or copper acetate gives rise to zinc or copper chlorophyll. These are green bodies, in which one atom of the metal occupies the position of magnesium in the parent chlorophyll. This reaction forms a very sensitive test for zinc or copper.

It is a matter of extreme difficulty to reintroduce magnesium into the phaeophytin molecule to form chlorophyll again. It has, however, been accomplished by Willstätter by means of the Grignard reaction, using methyl magnesium iodide.

Phaeophytin treated with hydrochloric acid in methyl alcoholic solution gives rise to methyl phaeophorbide, and this body when treated with concentrated hydrochloric acid gives phaeophorbide. Phaeophorbide prepared from chlorophyll *a* gives a substance known as phytochlorin *e* when allowed to react with alkali. The corresponding product for chlorophyll *b* is known as phytorhodin *g*. All these products contain no magnesium. When phaeophytin is treated with alkali it behaves as an ester and undergoes saponification, and the products obtained are nitrogen-containing acids, while two alcohols are removed from the molecule, methyl alcohol and an unsaturated primary alcohol phytol ($C_{20}H_{39}OH$). The same phytol is obtained from both chlorophylls. Phaeophytin *a* on treatment with dilute alkali gives rise to two isomeric products phytochlorin *f* and *g*, and these are also isomeric with

phytochlorin *e*, the corresponding products from phaeophytin *b* are phytorhodin *i* and *k*.

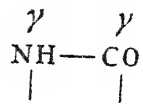
On treatment with alkali in the cold chlorophyll gives rise to salts of dibasic acids, the chlorophyllins. These are green bodies soluble in water. Treatment of chlorophyll with alkali does not remove magnesium from the molecule. Chlorophyllin when heated with alkali loses carbon dioxide and yields two isomeric acids: glaucophyllin, which is obtained at a temperature of 140° C., and rhodophyllin, which is formed at 165° C. Rhodophyllin, when still more strongly heated with alkali to a temperature of 200° C., loses a further molecule of carbon dioxide and gives a monobasic acid, pyrrophyllin. Pyrrophyllin, when heated with soda lime, loses a third molecule of carbon dioxide to give aetiophyllin, and this on treatment with acid gives a magnesium-free product, aetioporphyrin $C_{31}H_{36}N_4$. Aetiophyllin can be synthesized from aetioporphyrin by means of the Grignard reaction using methyl magnesium iodide. Chlorophyll *b* also gives rise to aetioporphyrin. Thus this product must be looked upon as the foundation of the chlorophyll molecule.

When treated with hot alkali, chlorophyll gives rise to iso-chlorophyllin. Iso-chlorophyllin further heated with alkali gives a series of products with loss of carbon dioxide, cyanophyllin, erythrophyllin and phyllophyllin. Phyllophyllin when heated with soda-lime gives aetiophyllin, and this on treatment with acid gives aetioporphyrin.

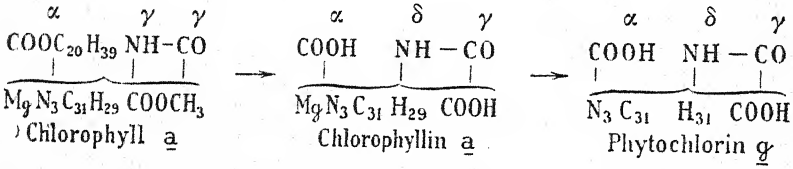
The aetiophyllin molecule contains magnesium but no oxygen, and it is therefore possible that the magnesium atom is directly attached to the nitrogen in the molecule.

When chlorophyll *a* and *b* are hydrolysed together by alkali, a series of colour changes from green to brown occurs. On the other hand, when hydrolysed alone, a different series of colours is formed. Thus chlorophyll *a* gives a green to yellow and chlorophyll *b* from green to red colour change. Then in a few minutes the original colour of the chlorophyll returns in alkaline media. The reaction creates the appearance of a complete decomposition and reformation of chlorophyll. This reaction is also given by the magnesium-free products of chlorophyll, e.g. phaeophytin. This is the only instance in which magnesium and magnesium-free compounds of chlorophyll assume the same appearance. The complex state of the magnesium in the molecule can be considered to be non-existent during the brown phase.

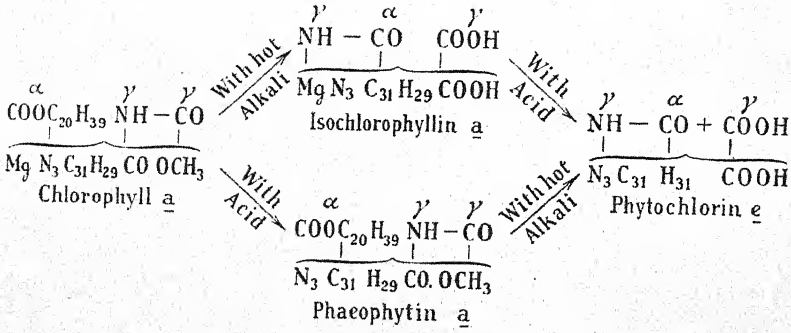
The reaction has been explained as follows: a group forming an essential part of the chromophoric complex is changed by hydrolysis and a new and similar group formed in its place. According to Willstätter, this change in colour is produced by the opening of a lactam ring in the original chlorophyll molecule. If this ring be represented by:



on relactamization the carboxyl may enter into union with another nitrogen group, which we can call δ-, or with the same nitrogen group. Relactamization may also occur in such a way that another carboxyl group, α-, unites, for example, with the nitrogen atom γ. In the case of chlorophyll *a*, on treatment with cold alkali it gives chlorophyllin *a*. Hydrolysis in the cold leads to the lactam ring being opened and the α-carboxyl which has been freed combines for the greater part with the δ-nitrogen atom. This occurs even when the α-carboxyl is already free:



When hydrolysed with hot alkali, the α-carboxyl which is liberated from the α-ester group, or which is already free, combines with the γ-nitrogen atom of the original lactam group:



Willstätter has suggested that at least three of the nitrogen atoms of the chlorophyll molecule can take part in this lactam formation, and since three carboxyl groups are also present, a very considerable number of lactams may be produced. This series of changes is spoken of as *allomerization*.

From the various reactions described above, we are in a position to write the formula of chlorophyll *a* as:

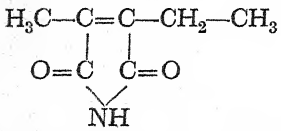
$$(C_{31}H_{29}N_3Mg)\ (CONH)\ (COOCH_3)\ (COOC_{20}H_{39})$$

and chlorophyll *b* as:

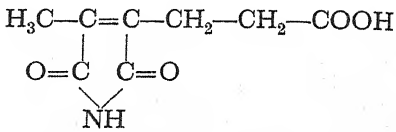
$$(C_{31}H_{27}N_3OMg)\ (CONH)\ (COOCH_3)\ (COOC_{20}H_{39})$$

In order to determine the structure of the basic part of the chlorophyll molecule, aetioporphyrin, oxidation and reduction of the various porphyrins has been carried out.

Phylloporphyrin on oxidation has given valuable information with regard to the ultimate constitution of chlorophyll. On oxidation it was found to give methylethylmaleinimide and haematinic acid:

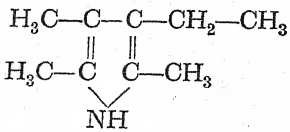


Methylethyl-maleinimide

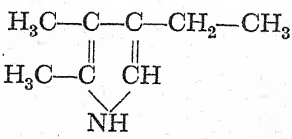


Haematinic acid

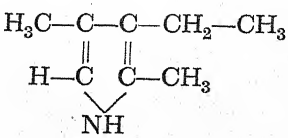
It was further found that the porphyrins on reduction yielded a mixture of pyrrole derivatives, ethyltrimethylpyrrole and two isomeric ethyldimethylpyrroles:



Ethyltrimethylpyrrole (phyllopyrrole)

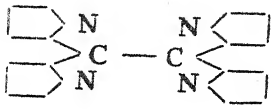


Ethyldimethylpyrrole (Isohaemopyrrole)

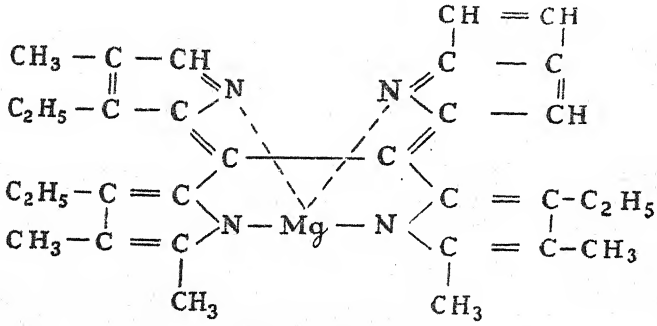


Krytopyrrole

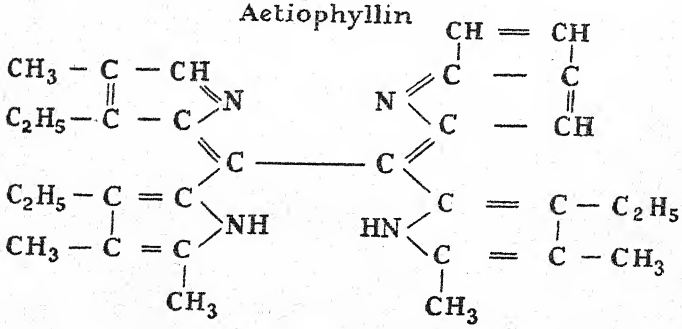
From the production of these various pyrrole derivatives by oxidation and reduction, Willstätter has concluded that aetioporphyrin is probably composed of four pyrrole nuclei. Deficiency of hydrogen leads to the assumption that double bonds are present, or alternatively that the pyrrole rings must be so arranged that eight atoms of hydrogen less are required than if single bonds be present. On these and other grounds Willstätter proposed the following framework for the aetioporphyrin molecule:



Arguing in this way, he provisionally assigned to actiophyllin and aetioporphyrin the following constitutions:



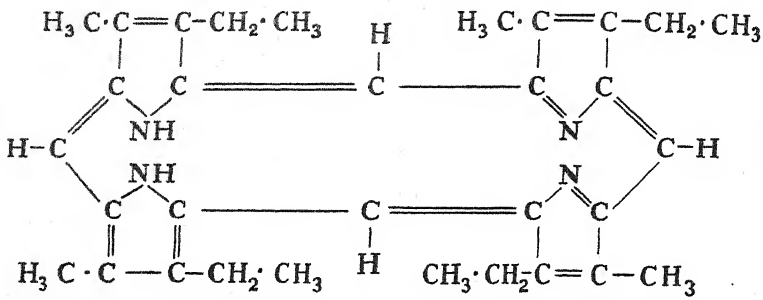
Aetiophyllin



Aetioporphyrin

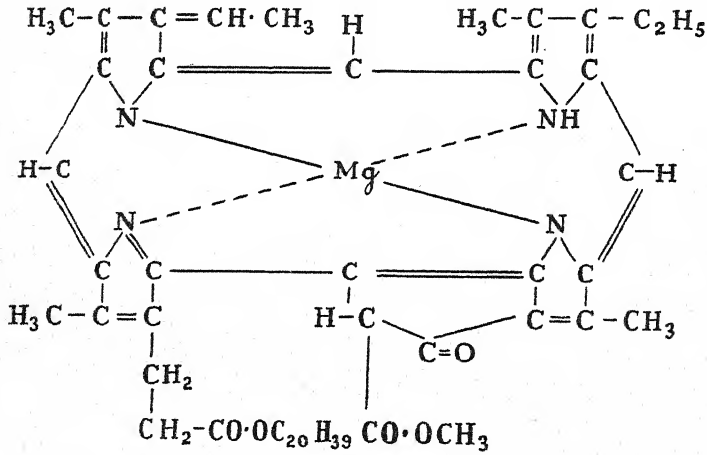
Hans Fischer and his co-workers in Germany, and Conant in America, have carried out further investigations on the constitution of chlorophyll which are too detailed to be included here.

Suffice it to say that Fischer has been able to synthesize a number of products closely related to aetioporphyrin as well as the latter product itself, to which he has given the constitution:



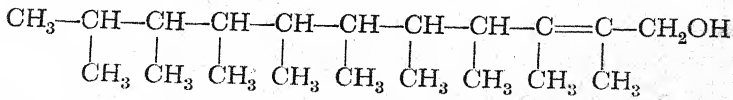
Aetioporphyrin

and has assigned the following constitution to chlorophyll *a*:



Chlorophyll a

Willstätter considered that the alcohol, phytol, produced by the hydrolysis of chlorophyll is an open chain product having the following constitution:



i.e. it is an unsaturated primary alcohol.

Phytol is a colourless, thick oil, soluble in most organic solvents. It boils at 147° C. under reduced pressure of 0·3–0·4 mm. of mercury. It readily undergoes oxidation and shows the presence of a double bond by the addition of two atoms of bromine or iodine.

In the course of their investigations on chlorophyll from a number of sources, Willstätter and Stoll found that the proportion of phytol obtained from different preparations showed considerable variation, whereas theoretically it should always be one-third the weight of chlorophyll. It was further ascertained that those plants which gave a low yield of phytol always gave a compound known as "crystalline chlorophyll." It was shown by Willstätter and Stoll that this result was due to the presence of an enzyme, chlorophyllase. This enzyme is active in alcoholic solution and hydrolyses the chlorophylls with removal of the phytol and replaces it in ethyl alcoholic solution with an ethyl radicle or in methyl alcoholic solution with a methyl radicle. These substitution products are called chlorophyllides. Chlorophyll *a* gives crystalline chlorophyll or ethyl chlorophyllide *a*

$$(C_{32}H_{30}ON_4Mg)\ (CO\ .\ OCH_3)(CO\ .\ OC_2H_5)$$

and methyl chlorophyllide *a* has the formula:

$$(C_{32}H_{30}ON_4Mg)\ (CO\ .\ OCH_3)(\ CO\ .\ OCH_3).$$

The chlorophyllides on treatment with acid give magnesium-free products, the phaeophorbides (see above). Chlorophyll can be synthesized once more from a solution of chlorophyllide. If an excess of phytol be added to an acetone solution of chlorophyllide, followed by the addition of dried and powdered leaves rich in chlorophyllase, chlorophyll is synthesized once more.

The Carotinoids.—The pigments associated with chlorophyll in the chloroplasts are collectively termed *carotinoids*. Carotin and xanthophyll are found in all chloroplasts, while in the Phaeophyceae there is an additional coloured compound, fucoxanthin.

Carotin.—This substance is a hydrocarbon with the formula $C_{40}H_{56}$. It is widely distributed in the plant world, other than in the chloroplasts. For example, it gives the reddish-yellow colour to carrots. The colour of tomatoes and red pepper is due to the presence of an isomer of carotin, lycopin, in the chromoplasts of these fruits.

Carotin is insoluble in water, but soluble in carbon disulphide,

chloroform, ether and benzene. It crystallizes in rhombohedra and quickly oxidizes in air to give a colourless product and absorbs 35 per cent of its weight of oxygen. It forms a very characteristic indigo-blue solution in concentrated sulphuric acid.

According to Karrer and others, carotin is composed of a mixture of two isomerides. One of these is optically active and has been designated α-carotin, while the optically inactive form has been called β-carotin.

Xanthophyll.—Whether xanthophyll is a single substance or a mixture of closely related isomeric and isomorphoric compounds has not been settled. The formula assigned to it by Willstätter is $C_{40}H_{56}O_2$ (cf. carotin).

It is insoluble in light petroleum, but readily soluble in chloroform and ether. It dissolves with difficulty in carbon disulphide and methyl alcohol. It absorbs oxygen like carotin, but bleaches more quickly. Its solution in concentrated sulphuric acid is deep blue.

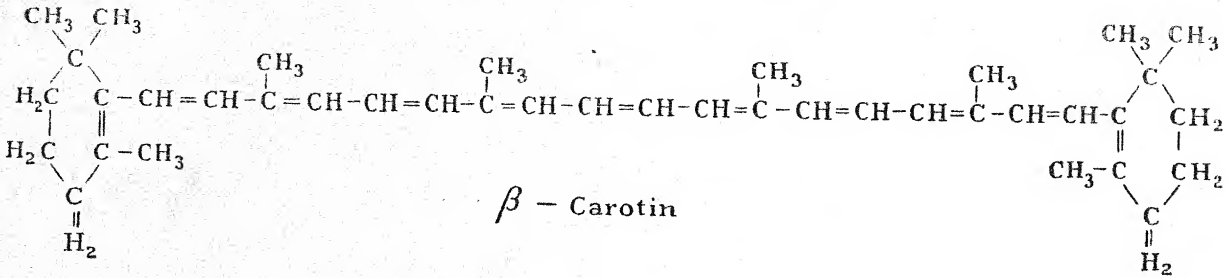
Fucoxanthin.—This compound was isolated from the Phaeophyceae by Willstätter and Page. It crystallizes in regular, dark-red hexagonal plates from acetone or methyl alcoholic solution. It is precipitated as needles from ethereal solution by the addition of light petroleum. It possesses the empirical formula, $C_{40}H_{54}O_6$. It is easily soluble in ethyl alcohol, but sparingly soluble in chloroform and ether and insoluble in light petroleum. The crystals of fucoxanthin do not readily bleach on exposure to air, but its solutions absorb oxygen to give complex substances containing a high percentage of oxygen. It readily combines with iodine in ethereal solution to give an iodide.

It possesses basic properties. When an ethereal solution of fucoxanthin is treated with 30 per cent hydrochloric acid, the ethereal layer is bleached and the acid layer shows a blue-violet colour, which on great dilution becomes sky-blue. This colour reaction is possibly due to the formation of an oxonium salt. It reacts with alkalis in alcoholic solution to give an additive compound which is easily dissociated, and at the same time the fucoxanthin is altered in some way. Willstätter considers that this behaviour is reminiscent of that of pyrone.

THE RATIO OF THE SINGLE PIGMENTS IN DIFFERENT PLANTS

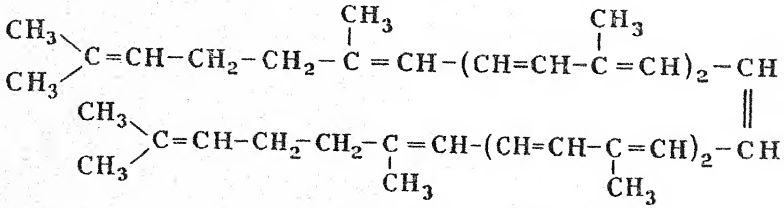
In the majority of seed plants the ratio of the two green pigments is almost 3 molecules of chlorophyll *a* to 1 of chlorophyll *b*. This

Karrer has proposed the following constitution for β-carotin:



β – Carotin

The constitution of α-carotin has not been definitely settled. Karrer has ascribed an open-chain structure for lycopin.



Lycopin

ratio is independent of photosynthesis and does not alter by day or by night.

Shade leaves as a rule possess more chlorophyll *b*. For the yellow pigments, the ratio has been found to be 1 molecule of carotin to approximately 2 molecules of xanthophyll. The tables given below show these various ratios. $Q\frac{a}{b}$ represents the ratio between chlorophyll *a* and chlorophyll *b*, $Q\frac{c}{x}$ that of carotin to xanthophyll and $Q\frac{a+b}{c+x}$ the ratio between the green and yellow pigments.

Component Ratio of Green and Yellow Pigments

Plant	*Living conditions*	$Q\frac{a}{b}$	$Q\frac{c}{x}$	$Q\frac{a+b}{c+x}$
Sambucus nigra	Sun-leaves	2·74	0·588	3·33
Sambucus nigra	Sun-leaves	2·83	0·629	2·83
Sambucus nigra	Sun-leaves	2·90	0·510	3·10
Aesculus Hippocastanum ..	Sun-leaves	2·89	0·699	2·84
Fagus sylvatica	Sun-leaves	3·13	0·653	3·45
Sambucus nigra	Shade-leaves	2·07	0·345	4·63
Aesculus Hippocastanum ..	Shade-leaves	2·30	0·353	4·70
Fagus sylvatica	Shade-leaves	2·92	0·553	6·02

In the green algae, the ratio of the pigments was found to be almost the same as that of the higher plants, but the Phaeophyceae were found to contain a higher amount of the yellow pigments. Among the yellow pigments, fucoxanthin was found to predominate.

Molecular Ratio between Chlorophyll and Yellow Pigments in the Phaeophyceae

Plant	$Q\frac{\text{Chlorophyll}}{\text{Carotin Xanthophyll Fucoxanthin}}$	*Carotin : Xanthophyll : Fucoxanthin*
Fucus	0·95	1·08 : 1 : 1·75
Dictyota ..	1·20	0·77 : 1 : 3·60
Laminaria ..	1·07	0·16 : 1 : 1·92

In the Phaeophyceae the chlorophyll is almost entirely composed of chlorophyll *a*.

THE CHLOROPLASTS

Next to the nucleus, the most conspicuous bodies that may be observed in plant cells are the plastids. These are bodies which have become specialized for certain specific physiological functions. The most important of these plastid bodies which we have to consider here are the chloroplasts. The chlorophyll pigments are not indiscriminately diffused through the cytoplasm of assimilating cells, but are confined to the chloroplasts, and as far as we know at present, photosynthesis centres round these bodies.

Chloroplasts vary in number in different plants. Some contain but one chloroplast to each cell (e.g. Anthoceros and Selaginella), others have two (Zygnema), whilst in the higher plants a considerable number occurs in each assimilating cell.

Chloroplasts show definite responsive movements to incident light, and Senn* has distinguished some eight types: (1) Mesocarpus, (2) Vaucheria, (3) Schistostega, (4) Eremosphera, (5) Streatella, (6) Funaria, (7) Spongy parenchyma and (8) Palisade parenchyma.

In Mesocarpus, in light of moderate intensity, the chloroplast, which is a broad plate, exposes its whole face to the incident rays. On the other hand, in intense light, only the edge is exposed. Vaucheria possesses a number of chloroplasts in its non-septate thallus. In moderate intensity of light the chloroplasts arrange themselves in rows on the upper side of the thallus, i.e. the side exposed to the light, whilst in intense light they move to the lower surface away from the light. The chloroplasts of Schistostega in moderate light are concentrated on the lower surface of the assimilating cells, while in strong light they move away to the sides. With moderate illumination, Eremosphera shows the distribution of the chloroplasts throughout the cytoplasm; whereas in strong illumination they collect round the nucleus. A similar condition is to be found in Streatella. Funaria exhibits the chloroplasts towards the surface of the cells in moderate light intensity at right angles to the incident rays, while in intense light they move away to the side walls. The chloroplasts of spongy tissue of the leaf behave in the same way as those of Funaria. The behaviour of the chloroplasts of palisade tissue has been carefully

* *Verhandl. naturforsch. Ges. Basel*, 1917, **28**, 104; *Zeit. f. Bot.*, 1919, **11**, 81.

investigated by Stahl, who found them entirely confined to the lateral walls, while the end walls were free. With change in intensity of incident illumination, the chloroplasts show changes in shape. In strong light they appear as flat discs, whereas in moderate light they can be seen to show protuberances towards the centre of the cell.

The actual structure of the chloroplasts and the manner in which the pigments are borne upon them are difficult points to determine. The body of the chloroplast, after removal of the pigments with alcohol, the so-called *stroma*, has been described as being composed of a fibrillar meshwork and the chlorophyll pigments as being held on the stroma in the form of minute droplets. The chemical nature of the stroma is not known. It is usually stated to be a protein, while lipins and fats are also said to be present.

According to J. H. Priestley and Irving* the pigments are confined to the periphery of the plastids, whereas Wager† has contradicted this statement and claimed that they were generally distributed throughout the plastid.

Zirkle‡ has studied the chloroplasts in the living cell. This method is much to be preferred to the use of "fixed" material, which may cause alterations in the structure of the chloroplasts. In monochromatic light of a known wave-length, it was found that in the light belonging to one of the light bands of the spectrum of the pigments they vanished. The tissue to be examined was first frozen at $-4°$ C., as it was found that this temperature did not affect the chloroplasts. Many water plants, for example, are embedded in ice throughout the winter. Below this temperature the chloroplasts seemed to liquefy and become spindle-shaped and eventually fuse into a green meshwork. With the sole exception of Selaginella, the various chloroplasts that were examined were found to be uniform in structure. The main study was made of the chloroplasts of the aquatic Elodea.

The chloroplasts of Elodea are found to be hollow, flattened ellipsoids. A central vacuole is present in which one or two starch grains may be present. The stroma contains numerous pores which connect the central vacuole with the cell cytoplasm and it is the presence of these pores which gives to the stroma its granular appearance. Surrounding each chloroplast there is a

* *Ann. Bot.*, 1907, **21**, 407.
† *Brit. Assoc. Rept.*, 1906.
‡ *Amer. J. Bot.*, 1926, **13**, 301, 321; 1927, **14**, 429.

more or less permanent sheath of non-granular cytoplasm. The pigments apparently are evenly distributed throughout the ground substance of the stroma, a result which confirms Wager's work, but contradicts that of Priestley and Irving. A certain amount of differentiation of chloroplasts was found, some, for example, contained little starch and were mainly concerned with photosynthesis, while in others much starch was discovered, and these apparently act as storage bodies.

The earlier workers, especially Schimper (1883) and Meyer (1883), claimed that plastids could not arise *de novo*, but could only be formed from pre-existing plastids. This is beyond doubt the case for the lower plants, such as mosses, liverworts and algae. In the higher plants, however, the matter is by no means on so secure a footing. Mitochondria or chondriosomes, which are small granules, threads or globules, nearly always to be found in cytoplasm, have been considered by a number of investigators to be the precursors of plastids in the higher plants. The matter, however, is still in a very controversial state.

Randolph* has described the development of the plastids for *Zea Mays* from minute primordia in the cytoplasm, and has called these structures "proplastids." According to Randolph, concurrently with the development and differentiation of the cells, these proplastids gradually enlarge, develop chlorophyll and become converted into chloroplasts.

THE CHEMISTRY OF PHOTOSYNTHESIS

The chemical reactions involved in the production of carbohydrates and the elimination of oxygen by the green plant in the presence of light have produced a large volume of work, but the problem still remains to be solved. In the course of the process, there is a conversion of radiant into potential chemical energy. It is clear that the process must take place in stages, the first stage being one of reduction. The central question that has to be answered is, Is this reduction of carbon dioxide brought about by photochemical means, or is it, as Warburg has suggested, due to the production of some substance in the cell which, under the influence of light, is capable of reducing carbon dioxide?

Photosynthesis is an endothermic reaction, i.e. a reaction in which heat is absorbed. Whatever the nature of the sugars formed

* *Bot. Gaz.*, 1922, **73**, 337.

in the course of the process, whether they be hexoses or disaccharides, they are substances with a higher potential energy than the initial compounds involved, carbon dioxide and water.

If photosynthesis were a purely photochemical reaction, then the Van't Hoff coefficient Q_{10} would be less than 2·0. Matthaei (see above) found the coefficient to be in the neighbourhood of 2·0. It is evident, therefore, that some other purely chemical reaction must also be involved as well.

It was suggested by Liebig in 1842 that organic acids might be the first formed products of photosynthesis and that these then suffered conversion into carbohydrates. Organic acids, e.g. oxalic, tartaric, malic, have been found in various plant tissues, notably succulents, and this was considered to lend credit to the theory. Their presence, however, is due to respiration (see Chapter XIII).

It was suggested in 1870 by the German chemist, Baeyer, that formaldehyde was the first substance produced in photosynthesis, and this by subsequent polymerization gave rise to sugars. The conversion of carbon dioxide and water to formaldehyde was considered by Baeyer to take place in the following stages:

$$CO_2 = CO + O$$
$$H_2O = H_2 + O$$
$$CO + H_2 = CH_2O$$
$$6CH_2O = C_6H_{12}O_6$$

The first stage in the reaction is the formation of carbon monoxide and oxygen. Carbon monoxide has only been found in the free state in one plant, the laminarian Nereocystis, and occurs here as a product of respiration. Bottomley and Jackson* claimed that *Tropaeolum majus* grown in an atmosphere of carbon monoxide lived and formed starch. This work has not been confirmed and has since been contradicted.

Formaldehyde can be obtained from leaves, and this fact was at one time considered to add confirmation to the formaldehyde hypothesis. It has, however, been shown by Jørgensen and Kidd† that the formaldehyde formed under such conditions is due to the oxidation of chlorophyll. In systems containing only carbon dioxide, water and pure chlorophyll, no formaldehyde is produced.

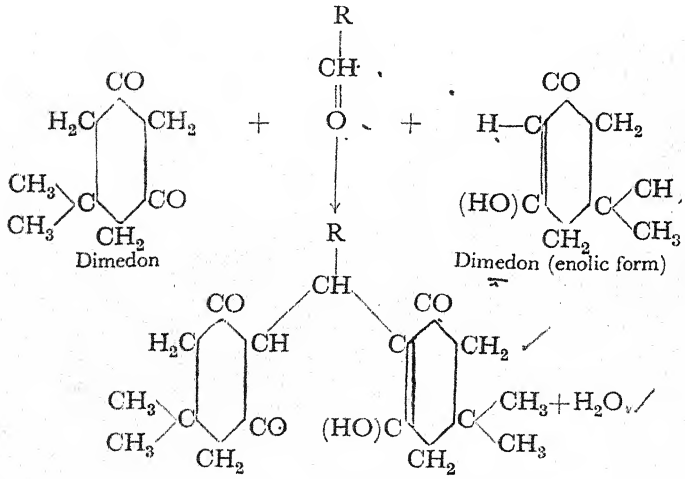
Baly and his co-workers have carried out a large number of

* *Proc. Roy. Soc.* (*Lond.*), 1904, **72B**, 130.

† Ibid., 1916, **89B**, 342.

in vitro experiments and claim to have substantiated the formaldehyde hypothesis. It must be pointed out in this connection that *in vitro* experiments can lead to very misleading results, since the plant is not a test tube, and secondly, that a large number of Baly's claims have been categorically contradicted.

Klein and Werner* have used the reagent dimedon (dimethylcyclohexanedione) to show that formaldehyde is a stage in photosynthesis. This reagent has been very successfully used by Neuburg in his investigations on the intermediate stages in alcoholic fermentation (see Chapter XIII), and Klein and Werner evidently had these results in mind when they employed this reagent. Dimedon forms condensation products with aldehydes.



With formaldehyde, formaldomedon is produced, and the crystals of this condensation product differ in melting-point and shape from those of the corresponding derivative of acetaldehyde, so that formaldomedon and acetaldomedon can be readily distinguished.

The plant mainly used in this investigation was *Elodea canadensis*, which was allowed to assimilate in nutrient solutions containing dimedon, and calcium bicarbonate was used as a source of carbon dioxide. Formaldomedon was subsequently found to be present in the external medium. If the plants were allowed to remain in the dark, acetaldomedon was found. Substances which are narcotic to living organisms, such as phenylurethane and potas-

* *Biochem. Zeit.*, 1926, **168**, 361.

sium cyanide, were found to prevent the formation of formaldomedon and only a small amount of acetaldomedon was discovered.

This work has been repeated by Barton-Wright and Pratt* on *Elodea canadensis*, who failed to confirm formaldehyde as a photosynthetic product. It was shown by these authors that bicarbonate solutions alone when exposed to light give rise to formaldomedon. In one case, it was ascertained that if a nutrient solution containing sodium bicarbonate and dimedon were exposed to light, as much formaldomedon was produced as when the plant was present.

Barton-Wright and Pratt have summarized the position of the formaldehyde hypothesis as follows: "Although the formaldehyde hypothesis has the merit of simplicity, which has probably been the prime cause of its wide popularity, no work has as yet convincingly shown that formaldehyde is produced normally in the green leaf or that it plays any part in the photosynthetic process of the living plant."

This statement is undoubtedly too sweeping. Since formaldehyde is toxic to living cells, it is hardly to be expected that it should accumulate to any marked extent in the free state under normal conditions. Presumably as soon as the carbonic acid is reduced, the formaldehyde formed, if it be formed, is immediately polymerized to sugar.

A number of other investigators have put forward theories with regard to the first substance formed in photosynthesis. Formic acid, glycollic aldehyde, $CH_2OH—CHO$, and other substances have been suggested as performing this rôle. There is no point in detailing these views, as they are not based upon sound experimental grounds and some are merely theoretical.

The views of Warburg,† however, are important in this connection. Warburg holds that there are several stages in the process. In the first stage, light is considered to act directly upon the chlorophyll (the so-called "photochemical primary reaction") to give rise to a "photochemical primary product." He considers that the rate of formation of this photochemical primary product is directly proportional to the amount of radiant energy absorbed per unit time. The next stage in the process is a reaction

* *Biochem. J.*, 1930, **24**, 1210.

† *Biochem. Zeit.*, 1919, **100**, 230; 1920, **103**, 188; 1926, **166**, 386. See also Warburg and Uyesugi, *Biochem. Zeit.*, 1924, **146**, 486.

between the photochemical primary product and an "acceptor." This acceptor is not carbonic acid itself, but is formed from it in the cell by a series of purely chemical reactions which take place independently of light.

The evidence on which these conclusions are based is, in the first place, that high concentrations of carbon dioxide and high light intensity do not increase the rate of photosynthesis with increase of these two factors, and secondly, that with rise of 10° C. of temperature the rate of photosynthesis is doubled. This is a characteristic of a chemical reaction. When light intensity is low, the Van't Hoff coefficient is near unity; this is a characteristic of photochemical reactions, and indicates that light is playing some rôle in photosynthesis. When light intensity is high, and $Q_{10} = 2 \cdot 0$, Warburg considers that some "dark reaction" or "Blackman reaction" is controlling photosynthesis.

Evidence in support of Warburg's view has been furnished by Li,* who showed that there is an initial inhibitory effect on the photosynthetic rate of a number of aquatics, when these were changed from a light of high available energy for assimilation to one of low available energy. In the same way, the reverse operation of removal from light of low available energy to one of high available energy produced an initial accelerating action on photosynthesis. The splitting off of oxygen is not considered to take place in the primary photochemical reaction but in a later and separate reaction, which may possibly be enzymic in nature.

The effect of various narcotics on the photosynthetic rate has been examined by Warburg, who found that potassium cyanide inhibited the use of atmospheric carbon dioxide by the plant for assimilation. When, however, the light intensity was reduced to such an extent that the rate of respiration exceeded the rate of photosynthesis, concentrations of potassium cyanide of 0·02M and lower did not affect the assimilatory rate. Under the experimental conditions, the carbon dioxide of the air was not used for photosynthesis, but, on the other hand, no carbon dioxide of respiration was released in the light, and according to Warburg, either this respiratory carbon dioxide or perhaps some intermediate product of respiration is used in photosynthesis. It was also found by Warburg that when a particular concentration of potassium cyanide inhibited the assimilatory rate by 50 per cent at high light intensity, a similar concentration will have no effect

* *Ann. Bot.*, 1929, **43**, 587.

at low light intensity. This result is taken to show that the secondary reaction between the photochemical primary product and acceptor is unaffected by potassium cyanide, but that it is the dark reaction or Blackman reaction that is inhibited by the cyanide.

In this connection, G. E. Briggs* and James† have also brought forward schemes to explain the mechanism of photosynthesis, without, however, committing themselves as to the nature of the first product or products formed. Briggs, for example, assumes that carbon dioxide combines with some substance S in the assimilating cells to give a complex S_c. This reaction is considered to be reversible. The nature of S is left vague; it may or may not be chlorophyll. The next stage in the process is the activation of the complex S_c by light to a new complex S_l. S_l is now broken down by a catalyst B to give the first carbohydrate of photosynthesis, oxygen and S once more. Briggs makes the further suggestion that the breakdown of S_l is brought about *via* the intermediate formation of a substance X formed from $S_l + B$. Thus according to Briggs we have the following reactions taking place:

$$S + CO_2 \rightleftharpoons S_c$$
$$S_c + \text{energy} \rightleftharpoons S_l$$
$$S_l + B \rightleftharpoons X$$
$$X \rightarrow B + S + \text{products.}$$

THE CARBOHYDRATES

It will be convenient to consider here some of the salient chemical characteristics of the carbohydrates. The name signifies that these substances are composed of carbon and water, i.e. hydrates of carbon, and the majority of compounds in this group of substances have the empirical formula:

$$C_x(H_2O)_y$$

The carbohydrates may be looked upon as ketonic and aldehydic alcohols.

The carbohydrates have been classified as Monosaccharides, Di-, tri-, and tetra-saccharides, and Polysaccharides. The monosaccharides have been further classified according to the number of carbon atoms in their molecules, thus: trioses (three carbon atoms), tetroses (four carbon atoms), pentoses (five carbon atoms), hexoses (six carbon atoms), heptoses (seven carbon atoms).

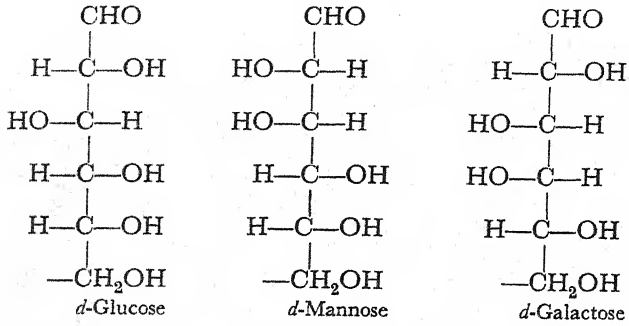
* *Proc. Roy. Soc. (Lond.)*, 1933, **113B**, 1.

† *New Phyt.*, 1934, **33**, 8.

Monosaccharides.—The sub-groups of the monosaccharides have already been enumerated above. The most important of these sub-groups as far as we are concerned are the pentoses and hexoses.

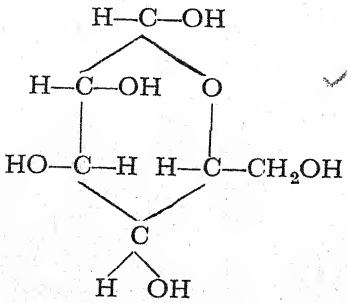
(*a*) *Pentoses.*—The pentoses have the formula $C_5H_{10}O_5$. It is doubtful if they exist in the free condition in plant cells. As pentosans (polysaccharides), condensation products with other sugars, they have a wide distribution in gums, mucilages, and pectic bodies.

(*b*) *Hexoses.*—The hexoses have the formula, $C_6H_{12}O_6$. There are sixteen possible stereoisomeric aldo-hexoses, all of which are known. Of these three have been found in plant cells, *d*-glucose, *d*-mannose and *d*-galactose:



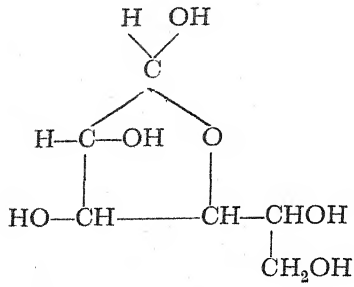
Of these three hexoses, only glucose has been found in the free condition in plant cells.

Glucose.—In solution, glucose has been shown by a number of recent investigations by Hawarth and his co-workers to have a lactone or internal anhydride structure. It was at one time thought that the ring structure of glucose was five-membered, but it has been clearly shown that in normal glucose the ring structure is six-membered:

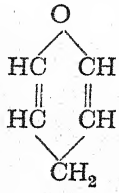


There is, however, evidence for the existence of a five-membered ring-structure, which represents a particularly active form of glucose, the so-called γ-glucose.

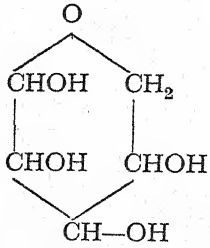
This labile form of glucose has been assigned the constitution:



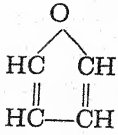
Hawarth has brought forward evidence to show that not only is this true of glucose, but also of other hexoses, e.g. galactose, fructose, and mannose, as well as the pentoses, arabinose, lyxose, and xylose. The six-membered ring compounds, or compounds with an amylene-oxide ring may be regarded as derivatives of pyranose, while the labile or active compounds, or compounds with a butylene-oxide ring, may be considered as being derivatives of furanose:



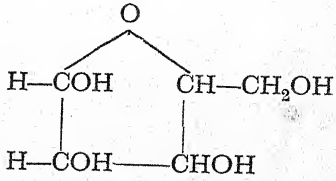
Pyran



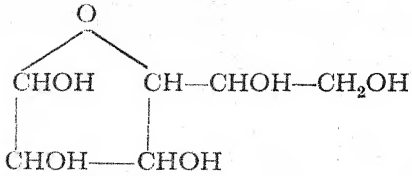
Pyranose form of aldopentose



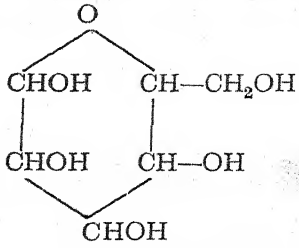
Furan



Furanose form of aldo-pentose



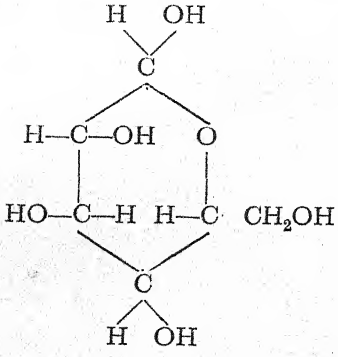
Furanose form of aldo-hexose



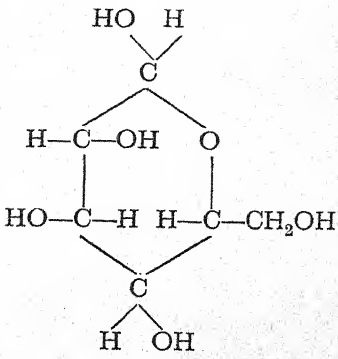
Pyranose form of aldo-hexose

When crystalline glucose is dissolved in water, it has been found that the optical rotating power of the solution exhibits changes for several hours, the rotation sometimes increasing, but more often decreasing, until finally equilibrium is reached. This power of changing rotation is termed *mutarotation*.

Such a result suggests that in solution there must be two optically active substances present, which are gradually changing from the one into the other. Actually, it has been shown that *d*-glucose exists in two isomeric forms, which differ in rotatory power. They are both dextro-rotatory, but one is more strongly dextro-rotatory than the other. These two isomeric forms have been isolated. If glucose only contained four asymmetric carbon atoms, the existence of these two isomers would be impossible; evidently *d*-glucose must contain five asymmetric carbon atoms. This has now been shown to be the case. These two isomerides are termed α-*d*-glucose and β-*d*-glucose and they possess the following structures:

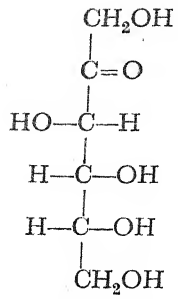


α-*d*-Glucose



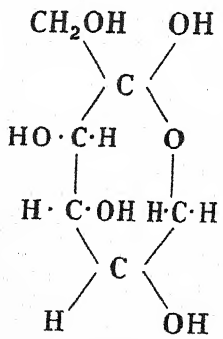
β-*d*-Glucose

Of the eight possible keto-hexoses, five are known, but only one occurs naturally, *d*-fructose:



d-Fructose

Actually, fructose is laevo-rotatory, but from its relationship to *d*-glucose it is termed *d*-fructose. Fructose, like *d*-glucose, occurs in the free condition in plant cells. It occurs in cane sugar and raffinose as a condensation product. The polysaccharide inulin is also a condensation product of fructose. As the free sugar it is present as a six-membered ring:



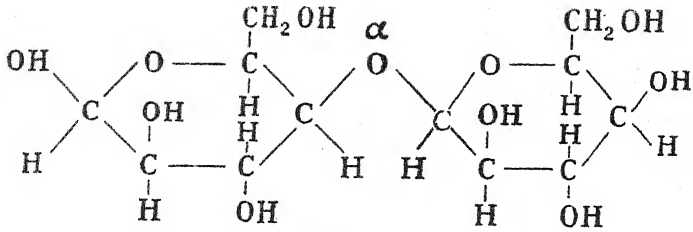
d-Fructose

In cane sugar, which is a condensation product of glucose and fructose, it is present in the labile or γ-form (see below).

Disaccharides.—The disaccharides formed by the condensation of two molecules of hexose possess the formula, $C_{12}H_{22}O_{11}$. The two hexoses in the molecule of disaccharide may be same or different. Disaccharides composed of one molecule of hexose and one molecule of pentose are also known.

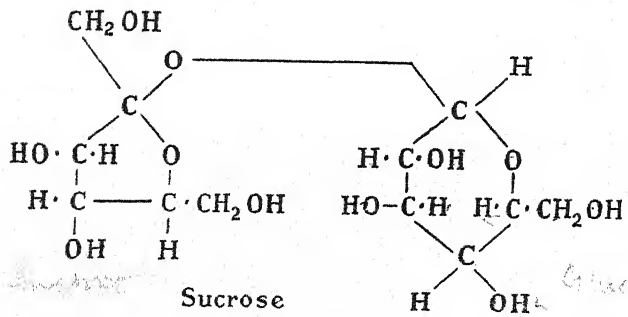
Maltose.—This is a hydrolytic product of starch. On hydrolysis

it gives glucose, and possesses the following constitution; i.e. it is an α-glucosido-4-glucose



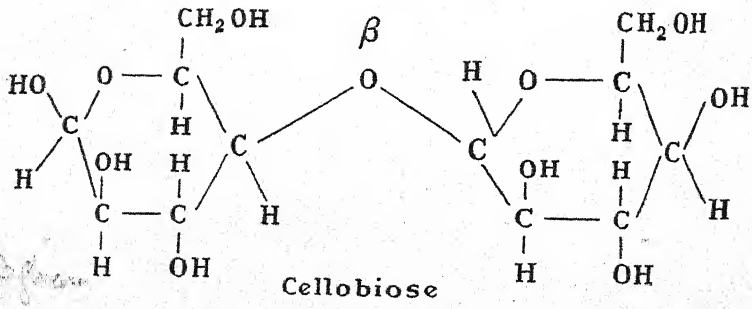
Maltose

Sucrose.—This disaccharide has a very wide distribution in plant tissues. On hydrolysis it gives rise to glucose and fructose. It possesses the following constitution:



Sucrose

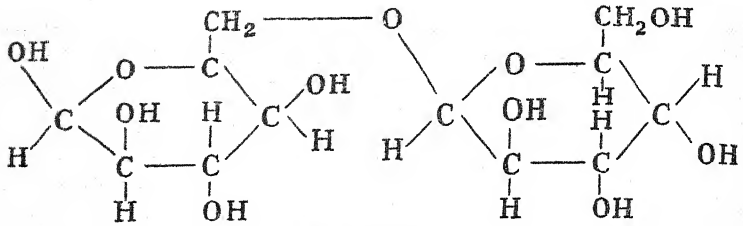
Cellobiose.—This disaccharide is obtained as an intermediate product in the hydrolysis of cellulose. It has been given the following constitution:



Cellobiose

i.e. it is a β-glucosido-4-glucose.

Melibiose.—This disaccharide has not been found in the free condition in plant tissues. It is obtained by the partial hydrolysis of the trisaccharide raffinose, and is a condensation product of two different hexoses, glucose and galactose:



Melibiose

Tri- and Tetrasaccharides.—Few trisaccharides have been found in plant cells. Gentianose occurs in the roots of *Gentiana lutea.* When hydrolysed by dilute acids it gives rise to glucose and fructose, whereas when the enzyme emulsin is used for this purpose, a mixture of glucose and sucrose is obtained. Raffinose is another trisaccharide that occurs in certain plants. It has been found in the sugar beet, barley grains and also in the seeds of the cotton plant. Hydrolysis with acid yields glucose, galactose and fructose, while with emulsin, galactose and sucrose are formed, and with invertase, melibiose and fructose. The tetrasaccharide, stachyose, has been found in the tubers of *Stachys tuberifera.* When hydrolysed it gives one molecule of glucose, one molecule of galactose and two molecules of fructose.

Polysaccharides.—The polysaccharides form a complex and important group of substances. They are formed by the condensation of a large number of molecules of monosaccharides, either pentoses alone or hexoses alone or a mixture of the two. When composed of pentoses alone, the complex is known as a *pentosan,* and similarly when composed of hexoses alone, the polysaccharide is called a *hexosan.* The pentosans possess the general formula $(C_5H_8O_4)_n$ and the hexosans $(C_6H_{10}O_5)_n$.

Starch and Cellulose.—In plants, starch is the most important reserve carbohydrate. It is a hexosan polysaccharide, and on hydrolysis with acid it gives rise to glucose. It occurs in plant cells in the form of characteristic stratified grains. Starch grains from different genera differ in their morphological and physical properties. The starch grain itself is a heterogeneous complex.

There is a central portion called amylose, which is soluble in water, and an insoluble outer layer or husk, termed amylopectin. Phosphoric acid has been found in chemical union in amylopectin. The starch grains obtained from grasses contain a further glucosan which has been called amylohemicellulose. This is a complex body containing calcium, magnesium, iron, and phosphoric and silicic acids. The phosphoric and silicic acids are apparently combined in the form of esters with the carbohydrate.

Cellulose is the main component of plant cell walls, although it is never present in the pure state but is mixed with other components such as mucilages, gums, etc. The cotton hair, which is an outgrowth from the seed coat of the cotton plant, is practically pure cellulose. Cellulose like starch is a glucosan. The chemistry of cellulose is complex and cannot be considered here, and the reader is advised to consult in this connection the monograph by Dorée, and for a general account of the components of the cell wall, Onslow's *The Principles of Plant Biochemistry*.

THE FIRST SUGAR OF PHOTOSYNTHESIS

We have already seen that by virtue of the chlorophyll pigments of the chloroplasts and in the presence of light, the green plant is able to synthesize carbohydrates from carbon dioxide and water. In 1862, Sachs demonstrated that starch is a product of photosynthesis and showed that it appeared in the light and disappeared in the dark. Sachs called starch the "first visible product" of carbon assimilation. But starch is not always produced in photosynthesis. It is used as a storage product in storage organs, e.g. tubers of potato, and in a number of plants it does not normally appear in the leaves as a result of assimilation. In the Gentianaceae, Umbelliferae, some Composites and a large number of monocotyledons, no starch is formed in the assimilating tissue.

It is improbable that starch is the first carbohydrate formed in photosynthesis, for it possesses a large and complex molecule and it is now known that simpler sugars precede its formation. The early work on the subject showed that leaves which did not produce starch as a result of assimilation formed considerable amounts of some substance or substances after exposure to light which reduced copper solutions. At the same time it was shown that substances were present that did not reduce copper solutions

unless they were first hydrolysed, and in 1883 Kayser demonstrated the presence of sucrose or cane sugar in the leaf of the vine. It was not, however, until 1893 that Horace Brown and Morris produced the first definite evidence that sugars were formed as a result of photosynthesis. They used for this purpose the leaves of *Tropaeolum majus* and discovered the presence of the two hexoses, *d*-glucose and *d*-fructose, and two disaccharides, maltose and cane sugar, but no pentoses were found to be present. All subsequent investigations have failed to reveal the *l*-isomerides of these sugars.

In 1914 Davis and Sawyer* claimed to have found pentoses to be present in the leaves of the mangold. Others, however, have denied the presence of pentoses in leaves. Davis and Sawyer used the Kröber method for estimating pentoses. In this method the material is distilled with 12 per cent hydrochloric acid and the furfural evolved is collected and estimated as the insoluble phloroglucide. The method, however, is inaccurate in the presence of hexoses. Moreover, "uronic" acids, which are carbohydrate derivatives with an aldehydic and carboxylic group in the molecule, also give furfural on distillation with 12 per cent hydrochloric acid, and these are widely distributed in plant tissues. Davis, Daish and Sawyer† were unable to discover the presence of maltose and consider that its presence in Brown and Morris' material may possibly have been due to hydrolysis of starch by diastase in the leaf cells, as the method of killing employed was not sufficiently rapid to bring enzyme action to an immediate halt.

It can definitely be said, at the present time, that the products of photosynthesis, either direct or indirect, are glucose, fructose, cane sugar and starch. The question arises here: Which of these substances is first formed in assimilation? Unfortunately, the question is not yet satisfactorily settled. On purely chemical grounds it is to be expected that hexoses, either glucose or fructose, or both together, are the first sugars formed in the leaf in photosynthesis. Nevertheless, until comparatively recent times, the major number of investigations on this subject reported cane sugar as the first sugar of photosynthesis.

Such a problem as this is difficult to investigate owing to the number of diverse factors that enter into it. In the first place, the experimental technique is difficult. The quantities of these substances present in the leaf are small, and unless great care is

* *J. Agric. Sci.*, 1914, **6**, 406.

† Ibid., 1916, **7**, 255.

exercised in their isolation and subsequent manipulation they may suffer conversion into other products. In the second place, the rates of change of these various products may be very different, and respiration and translocation further complicate an already complicated problem. Lastly, the interpretation of the experimental data, when they have been obtained, is surrounded with difficulties.

Two further points must be kept in mind in this connection, namely: the so-called up-grade sugars, which are directly formed in photosynthesis from carbon dioxide and water, and the down-grade sugars formed by the hydrolysis of reserve carbohydrates, such as starch.

It has already been mentioned that Horace Brown and Morris investigated quantitatively the sugars present in Tropæolum leaves and used for their purpose detached as well as normal leaves. The data from one of their experiments are given below:

Analyses of Carbohydrates in Leaves of Tropaeolum

(Results Expressed as Percentage of Dry-Weight of Leaf)

Carbohydrates as Percentage Dry-Weight of Leaf	*Picked from Plant at 5 a.m.*	*Detached Leaves in Water, Insolated until 5 p.m.*	*Picked from Plant at 5 p.m.*
Starch	1·23	3·91	4·59
Sucrose	4·65	8·85	3·86
Glucose	0·97	1·20	0·00
Fructose	2·99	6·44	0·39
Maltose	1·18	0·69	5·33

It will be seen from these figures that starch accumulates in the leaves after they have been exposed to light. In the detached leaves, translocation is prevented, and the total sugars increase over normal leaves, the greatest increase being found in the cane sugar and fructose fractions. It will be seen that no glucose was found after the leaves had been allowed to assimilate all day, and the fructose was very small in amount. In another experiment, in which detached leaves were kept with their petioles in water in the dark for 24 hours, the following values were recorded:

Analyses of Carbohydrates in Leaves of Tropaeolum

(Results Expressed as Percentage of Dry-Weight of Leaf)

	Leaves Picked and Dried at Once	*Leaves Picked and Placed with Petioles in Water in Dark for 24 Hours*
Starch	3·69	2·98
Sucrose	9·98	3·49
Glucose	0·00	0·58
Fructose	1·41	3·46
Maltose	2·25	1·86

It can be seen from these figures that when detached leaves are allowed to remain in the dark, there is on the whole a decrease in the sugars, the loss being mainly in the cane sugar fraction. The fructose fraction, on the other hand, shows an increase, while there is no significant change in the glucose fraction.

Brown and Morris argued from these results that sucrose and not glucose was the first sugar of photosynthesis, since the sucrose increases in amount on exposure to light and the glucose does not increase under such conditions. The cane sugar was considered to accumulate until a certain concentration was reached, when excess was converted into starch, while for purposes of translocation, cane sugar is hydrolysed to glucose and fructose, and removed as such. The fact that fructose was discovered to be in excess of glucose was considered to be due to the fact that glucose is used in respiration to a greater extent than fructose.

The presence of maltose in leaves has been disputed. It has already been stated that Davis, Daish and Sawyer claimed that the maltose found to be present by Brown and Morris was due to the hydrolysis of starch by diastase, since the method of killing the material, namely, by heating it to 100° C. in an oven, would not bring about instantaneous death.

A careful investigation by Parkin* of the sugars of the Snowdrop, *Galanthus nivalis*, also led this investigator to the view that sucrose and not hexose is the first sugar of photosynthesis. By employing a monocotyledonous leaf like the Snowdrop, Parkin did away with the complication caused by the presence of

* *Biochem. J.*, 1912, 6, 1.

starch, which only occurs in the leaves to a negligible extent in the guard-cells of the stomata.

In the early part of the growing season, sucrose was found to be in excess of hexose in the leaves, but later in the season the reverse situation was found to occur. Parkin found that during any day of the spring, no matter at what hour the sugars were determined, the amount of hexose sugars remains fairly constant in value, whereas the sucrose fluctuates greatly. Moreover, the sucrose values fall during the night and increase during the day, while detached and insolated leaves were found to contain definitely more sucrose than the controls, yet the hexose concentration remained more or less constant. Some of the values obtained by Parkin are given below:

Analyses of Carbohydrates of Leaves of Galanthus

(*Results Expressed as Percentage of Dry-Weight of Leaf*)

Time of Estimation	*Sucrose*	*Hexose*	*Total Sugars*
9 a.m. ..	11·22	6·35	17·57
3.30 p.m. ..	14·65	5·48	20·13
	+ 3·43	− 0·87	+ 2·56
8.15 a.m. ..	8·88	9·40	18·28
4.15 p.m. ..	12·92	10·74	23·66
	+ 4·04	+ 1·34	+ 5·38

The investigations of Davis, Daish and Sawyer, which have already been mentioned, were large-scale determinations of the carbohydrates present in the leaves of the potato and mangold, in order to discover whether hexose or sucrose is the first sugar of photosynthesis.

The carbohydrates estimated were glucose, fructose, sucrose, maltose, starch, pentoses, and pentosans. The material was killed by throwing it into boiling 95 per cent alcohol. Collections of leaves were made at two-hourly intervals over twenty-four hour periods. Three "experimental runs" were carried out on the mangold, the first in August, the second in September and the third in October. In the mangold leaf, starch was found to be

present during the early stages of growth, but was absent later. They were unable to find the presence of maltose. The results were calculated as a percentage of the dry-weight and the sugars of lamina and petiole were treated separately.

In the August experiment, it was found that soluble sugars, both hexoses and sucrose, increased in the light to a maximum about noon and then fell away until the following dawn. The sucrose was found to be in excess of hexose, while the pentosan concentration remained fairly constant. In the September and October experiments, hexose was found to be in excess of sucrose.

Davis, Daish and Sawyer, like Brown and Morris and Parkin, interpreted their data as showing that sucrose was the first sugar of assimilation. It is probable that the sampling error in this work was high. These investigators attempted to escape from this difficulty by using large samples for each individual determination, but even so, they have recorded values for sucrose by optical rotation and copper reduction methods that differ by as much as 20 per cent.

According to Davis, Daish and Sawyer, the sucrose first formed in photosynthesis is hydrolysed to glucose and fructose prior to translocation, and it is as hexoses that sugars are carried to the storage organs, where they are synthesized to sucrose once more. Of the various authors who have claimed that sucrose and not hexose is the first sugar of assimilation, Davis, Daish and Sawyer are the only ones that put forward a possible suggestion as to how this may come about: "It would seem indeed, that plant leaves in general possess in the chloroplasts a mechanism for elaborating cane sugar directly from the carbon dioxide of the air."

Examination of the curves obtained by Davis and his co-workers does not give any conclusive evidence that sucrose is the first formed sugar in the leaf. The data can be equally well interpreted on the view that hexose precedes sucrose formation. From the purely chemical standpoint it is unlikely that sucrose should be formed first and then hydrolysed back to glucose and fructose. The fact that has been most insisted upon by upholders of the sucrose interpretation, namely, that hexose remains relatively constant during the period of assimilation whereas sucrose fluctuates markedly, can be explained on the grounds that there is a state of equilibrium between hexose and sucrose:

$$\text{Hexose} \rightleftharpoons \text{Sucrose}$$

When the concentration of hexose reaches a certain value, it is converted into sucrose. Thus the concentration of hexose remains relatively constant in amount. If the concentration of hexose should fall, then the sucrose will be hydrolysed back to glucose and fructose, so that the hexose still remains constant in amount.

The question of which sugar is first formed in assimilation has been investigated from a different angle by Weevers.* Instead of using plants with green leaves, he employed for his purpose species with variegated leaves and determined the sugar-content of the green and non-green portions. Among the plants used in this work were *Acer Negundo*, *Hedera Helix*, *Humulus Lupulus*, *Pelargonium zonale*, *Cornus sanguinea* and *Aesculus Hippocastanum*. With the exception of *C. sanguinea* and *A. Hippocastanum*, sucrose alone was found to be present in the non-green areas, whereas both hexose and sucrose were present in the green portions of the leaf. In *Cornus sanguinea* and *Aesculus Hippocastanum* hexose was also present in the non-green parts, but only in very small amount. Since the non-green parts of these leaves, owing to lack of chlorophyll, are unable to photosynthesize, it is a significant fact that no hexose was present. A further experiment, however, gave still more conclusive evidence that hexose and not sucrose is the first sugar of carbon assimilation. It was ascertained by Weevers that the leaves of a variegated variety of *Pelargonium zonale*, when kept in the dark for a period of forty-eight hours, were completely deprived of all sugars. At the end of this time the plants were exposed to light, when it was found that hexose and not sucrose was first formed in the leaves. The results of this experiment are tabulated below:

Analyses of Leaves of Variegated Form of Pelargonium zonale

After ½ hour insolation (per 10 gm. dry-weight)	Traces of reducing sugars. Sucrose absent
After 1 hour insolation 0·3 per cent hexose ..	Traces of sucrose
After 3 hours insolation 0·4 per cent hexose ..	0·3 per cent sucrose
After 5 hours insolation 0·6 per cent hexose ..	0·3 per cent sucrose

From these results there can be little doubt that hexose and not sucrose is the first sugar of photosynthesis.

Barton-Wright and Pratt† have examined the sugars of the

* *K. Akad. Wetensch. Amsterdam Proc. Sci.*, 1924, **27**, 46.
† *Biochem. J.*, 1930, **24**, 1217.

leaf of the daffodil (*Narcissus Pseudo-Narcissus*) in an attempt to discover whether hexose or sucrose is the first sugar of photosynthesis. Like Parkin, these authors chose Narcissus on account of the fact that no starch is present in the leaves, except in small amounts in the guard-cells, and this factor therefore does not complicate the problem. The results were expressed as a percentage of the "residual dry-weight," i.e. dry-weight less total sugars, since this value gives a more constant basis for calculations of this nature, the major fluctuations in dry-weight of an organ being due to fluctuations in carbohydrates. It would therefore seem to be more reasonable to make the assumption that the remaining fraction of the dry-weight will be relatively constant over *short* periods of time. Samples were taken at hourly intervals as it was considered that this would give a better picture of the fluctuations of the sugars of the leaf. The sampling error was small, 2 per cent. The sugars of the leaf were determined at different times during the growing season (March 31st, May 8th, May 20th).

The determinations carried out on March 31st gave no conclusive evidence as to whether hexose or sucrose was the first sugar of assimilation. It was considered that it is improbable that sucrose is the first sugar, for it was discovered that the maximum values of sucrose were only attained after a number of hours of assimilation. This series of determinations confirmed the earlier observations of Parkin on *Galanthus nivalis* that in the early part of the growing season sucrose is in excess of hexose.

In the second series of observations, which was only carried out for a short period of time (four hours), and was made after the flower buds had opened, it was found that at this stage in the growing season hexose was in excess of sucrose: a result again in agreement with Parkin and others. The plants were first darkened for a period of twenty-four hours and the determinations were commenced at 10 a.m. The results are shown in Fig. 25 A. In the first hour hexose rose and sucrose fell, but from 11 a.m. onwards there was continuous rise in sucrose until the close of the experimental period. Between 11 a.m. and noon there was heavy rain and a decrease in light intensity, and during this period a heavy fall in hexose was recorded, whereas the sucrose rose continuously throughout this time. Although this experiment gave definite evidence for hexose being the first formed sugar in the leaf, the last series of determinations carried out on May 20th gave still

further support for hexose being the first sugar formed in the leaf.

In this last series of determinations, which were carried out over a period of nineteen hours, continuous records were kept of humidity, temperature and stomatal aperture. The results, graphically expressed, are shown in Fig. 25 B. The data were also statistically examined and it was found that the correlation coefficient between hexose and sucrose was significant

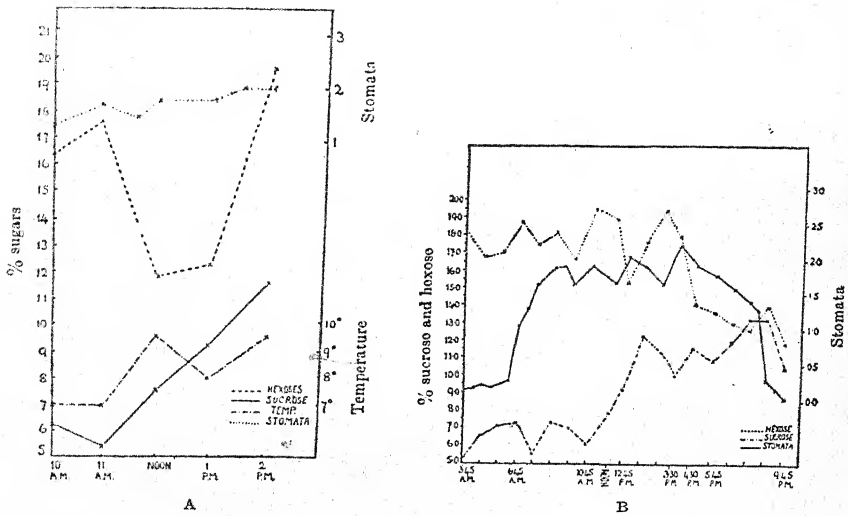


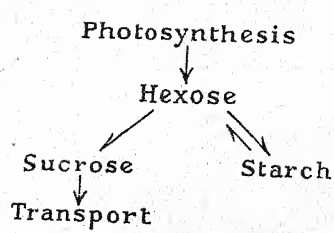
FIG. 25.—A. Curves showing variations in hexose and sucrose in the Narcissus leaf over a period of four hours. During a rainy spell the hexose fell, but sucrose remained unaffected. B. Fluctuations in hexose and sucrose over a period of nineteen hours in Narcissus leaf. It should be noticed that the curve for sucrose only slowly rises to a maximum. (After Barton-Wright and Pratt.)

($r = +0 \cdot 7100$). The correlation between temperature and hexose ($r = +0 \cdot 4700$) was not quite significant, but the coefficient was higher than that between temperature and sucrose ($r = +0 \cdot 3000$). It was further ascertained that there was negative drift with time in the case of hexose (hexose/time, $r = -0 \cdot 8400$), whereas there was a positive drift with time for sucrose values (sucrose/time $r = +0 \cdot 8750$). If the curve for sucrose be examined in Fig. 25 B it will be seen that the sucrose percentages only begin to rise continuously after 10.45 a.m., in other words, increase in sucrose lags behind increase in hexose. In order to determine whether or not this lag was significant,

these authors shifted the values on between hexose and sucrose, and found that there was an increase in the value of the correlation coefficient. When the hexose values were shifted on a period of four hours, the correlation rose from $r = +0 \cdot 7100$ to $r = +0 \cdot 7700$. Variations in hexose are therefore reflected in sucrose and the lag period is significant.

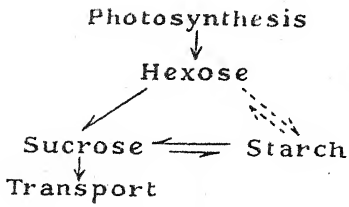
It was considered by these authors that these data, taken as a whole, support the view that hexose and not sucrose is the first sugar of the leaf. They point out that first of all there is a significant correlation between hexose and sucrose; secondly, the correlation between hexose and temperature is higher than that between sucrose and temperature; thirdly, there is a negative drift with time in the hexose values and a positive drift with time in the sucrose percentages, and lastly, increase in sucrose lags behind increase in hexose.

An examination of the carbohydrates of the potato leaf have been carried out by Barton-Wright and McBain.* In this case, also, the evidence was considered to support the view that hexose is the first sugar formed in the leaf. In the early part of the growing season it was found that sucrose is in excess of hexose, while later the reverse is the case. Much the same data were relied upon by these investigators as those given for Narcissus. A lag was discovered between increase in hexose and increase in sucrose, there was negative drift with time for the hexose values and a positive drift with time for the sucrose values. It was also discovered that increase in sucrose was dependent upon decrease in the hexose concentrations, and the reverse reaction does not occur to any great extent. In the case of the potato, the correlation between temperature and hexose was found to be significant, while that of temperature and sucrose was not. In certain conditions it was found that starch could be formed from sucrose. In light of high intensity it was considered that the following reactions occur:



* *Trans. Roy. Soc.* (*Edin.*), 1932, 57, 309.

whereas in light of low intensity, the inter-relationships between the carbohydrates were thought to be:



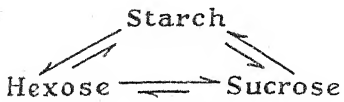
It will be seen from these schemes that starch may be formed from two different sources, (*a*) from hexose or (*b*) from sucrose. It has been known for a considerable time that when leaves are floated on various sugar solutions in the dark, starch formation can be induced. Of the different sugars that have been used for this purpose, such as glucose, fructose, galactose and sucrose, sucrose has been found to be the best starch former.

The problem of starch formation from sucrose has been investigated by de Wolff,* who showed that if slices of potato tuber were artificially dried, there is an increase in the sugar-content, and he was able to show that this increase was directly due to sucrose, the hexose-content remaining approximately constant. It was thought by de Wolff that the conversion of sucrose to starch is a reversible reaction, but that it takes place through several stages. It was considered by Barton-Wright and McBain that in the lamina of the healthy potato leaf, the reaction between starch and sucrose takes place principally in the direction starch→sucrose. Recent investigations on the question of sugar transport in the higher plants have shown that sucrose is apparently the principal form in which sugar is translocated (see Chapter XII). If this view be correct there will be a continuous flow of sucrose out of the lamina to the developing tubers in the potato plant, and it may be that it is on this account that the concentration of sucrose does not reach the necessary level for the reverse reaction, sucrose→starch, to take place to any great extent in the lamina.

When the potato is attacked by a virus disease known as "leaf-roll," it has been found that the leaves become choked with starch. This disease brings about necrosis of the phloem, the principal channel of transport in the plant for elaborated food

* *Biochem. Zt.*, 1926, **176**, 225; **178**, 36.

material, and of necessity the metabolic products elaborated in the lamina of the leaf cannot be removed normally. It has been found by Barton-Wright and McBain that in potato plants suffering from leaf-roll, the relationship between sucrose and starch is very close. It was found by these authors that in the early stages of the disease the rate of photosynthesis is very small and the following reactions are taking place in the lamina of the leaf:



There is hydrolysis of starch to hexose and condensation of hexose to sucrose, and finally the sucrose is converted back to starch once more.

H. F. Clements,* who has investigated the formation of carbohydrates over twenty-four hour periods in the potato, *Helianthus annuus* and *Sojamax*, is also of the opinion that "simple" sugars (hexoses and pentoses) are formed before sucrose in the leaf. The arguments advanced by this investigator are similar to those of Barton -Wright and Pratt.

If a hexose is the first sugar of photosynthesis, there is another complication that has to be considered. Sucrose is a condensation product of the two hexoses, glucose and fructose, and the question arises here, Is glucose first formed from carbon dioxide and water and then a part converted into fructose, or are both formed simultaneously in the leaf? It has been shown that if aqueous solutions of various hexoses, such as *d*-glucose, *d*-fructose, and *d*-mannose be left in the presence of a 0·05 equivalent of calcium hydroxide or di-sodium hydrogen phosphate, a mixture of hexoses composed of *d*-mannose, *d*-glucose, *d*-fructose, *d*-pseudofructose as well as α-, β-, and *d*-glucose is obtained.

If glucose is first formed in the leaf cells the plant may possess some mechanism, which we do not understand at present, to convert a part of this glucose into fructose and other hexoses. Free mannose has not been discovered in the leaf. Clements† has conducted an investigation on forty-two different species of plants of various kinds, including angiosperms, gymnosperms and pteridophytes, and in no case found free mannose to be present. Its detection is simple, for it is the only known hexose that forms

* *Bot. Gaz.*, 1930, **89**, 241.

† *Plant Physiol.*, 1932, **7**, 567.

a sparingly soluble hydrazone. It has been suggested by Clements that fructose is formed independently of glucose, from carbon dioxide and water during photosynthesis. If this were not the case it would be expected that mannose would also be present, unless the stereochemical relationships known to exist between these three hexoses are different in the cell sap from what they are in pure water.

CHAPTER IX

THE FATS

THE fats are of the greatest physiological importance to the living organism. In the animal economy they are the chief form in which excess of food material is stored. Plants also make use of fats as a food reserve, especially in seeds and spores.

In an animal, during starvation, the carbohydrate supplies are soon exhausted, and reserve fats are then utilized to supply energy. Gram for gram, the fats on combustion give more than twice the amount of heat of carbohydrates. Thus, on combustion one gram of carbohydrate gives 4·1 large Calories of heat, whereas one gram of fat gives 9·1 large Calories.

The fact that during starvation an animal draws upon its reserve supplies of fats, is in itself a matter of great importance, but recent lines of investigation have shown that the fats have a more extensive field of significance in the physiological activities of the cell than has been realized heretofore. The chemical definition of a fat is an ester of the trihydric alcohol glycerol and fatty acids, but if the term "fats" be extended to all substances containing fatty acids or with which fatty acids are associated in the cell, the group becomes one of first importance in physiology.

If this wider application be adopted for fats, another term is necessary to include all "fats" of physiological importance. The term "lipide" has been suggested by the International Congress of Applied Chemistry and the name lipide is now widely used in America in this connection.

Bloor* has suggested the following classification for substances to be included under the term lipide:

(1) They are soluble in fat solvents, such as chloroform, benzene and ether, but insoluble in water.

(2) Their relationship to the fatty acids is that of an ester, either potential or actual.

(3) They are utilized by living organisms.

(A) *Simple Lipides.*—These are esters of fatty acids with various alcohols.

(i) Fats. Esters of fatty acids and glycerol.

* *Physiol. Rev.*, 1922, **2**, 92; *Chem. Rev.*, 1925, **2**, 243.

(ii) Waxes. Esters of fatty acids and alcohols other than glycerol.

(B) *Compound Lipides.*—In this group the esters of the fatty acids contain additional groups to an alcohol and fatty acid.

(i) Phospholipides or Phosphatides. These are substituted fats containing phosphoric acid and nitrogen. Examples are lecithin, kephalin and sphingomyelin.
(ii) Glycolipides. The glycolipides are compounds of a carbohydrate with fatty acids. They contain nitrogen, but no phosphoric acid. The so-called cerebrosides are glycolipides.
(iii) Indefinite group of substances which are not well characterized for classification, such as aminolipides, sulpholipides, etc.

(C) *Derived Lipides.*—This group includes substances derived from groups (A) and (B) by hydrolysis.

(i) Fatty acids of various series.
(ii) Sterols and other alcohols of high molecular weight which are often found in combination with fatty acids and which are soluble in fat solvents. Examples are cholesterol ($C_{27}H_{45}OH$), ergosterol ($C_{27}H_{41}OH$) and coprosterol ($C_{27}H_{47}OH$).

There is one general feature common to the lipides namely, their solubility in the so-called fat solvents. There is, however, variation shown by the group in this respect, thus lecithin is insoluble in acetone, while kephalin is practically insoluble in alcohol, and the cerebrosides are soluble in ether with difficulty.

A matter of considerable interest is the melting and solidifying points of the lipides. It has been found that beef fat melts at 49·5° C., but solidifies at 36° C. In other words the melting-point is considerably higher than the temperature at which solidification takes place again. This peculiarity has been discovered in a number of other glycerides. If, however, the cooling of the liquid fat be carried out very slowly, the melting-point and solidification point agree very closely. According to Adam, who has developed a technique for preparing thin films of fatty acids and their esters on water, liquid or solid films may be obtained from one and the same compound, the particular physical condition depending on the pH of the water. Thus, he was able to show that palmitic

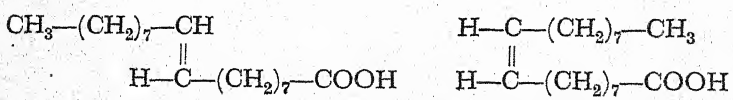
acid, which melts above 62·6° C., will form a liquid film at ordinary temperature if the pH of the water be 5 or lower. If a lateral pressure of sufficient magnitude be now applied, the liquid film will solidify. This work may have some bearing on the fact that in living cells lipides occur as minute liquid droplets at the ordinary temperature and in a predominantly aqueous medium.

THE FATTY ACIDS

The acids which have been obtained by the hydrolysis of lipides belong to several different series. We have first of all the saturated acids of the general formula, $C_nH_{2n}O_2$, where n varies from 2 to 30. The simplest member of this series is acetic acid.

The unsaturated acids belong to several different series, depending on the number of double bonds present in the molecule.

(1) The first series contains only one double bond and has the general formula $C_nH_{2n-2}O_2$. This is known as the oleic series. To it belongs oleic acid ($n = 18$) which gives this series its name, hypogeic acid with $n = 16$, gadoleic acid, $n = 20$ and erucic acid, $n = 22$. In this group isomers are known in which the double bond may occur in different positions in the molecule. Another kind of isomerism is also exhibited, namely the so-called *cis-trans* isomerism. Thus normal oleic acid has an isomer elaidic acid, which may be prepared from the former by treatment with fumes of nitrous acid. The difference in the constitution of these two substances may be expressed thus:



It is not known, however, which of these constitutions applies to oleic and which to elaidic acid.

(2) The next series of unsaturated fatty acids contains two double bonds in the molecule and possesses the general formula, $C_nH_{2n-4}O_2$. This is called the linoleic series from linoleic acid ($n = 18$).

(3) The third series of unsaturated acids contains three double bonds in the molecule. This is called the linolenic series from linolenic acid and has the general formula, $C_nH_{2n-6}O_2$. In linolenic acid itself, $n = 18$.

(4) and (5). Two other series of unsaturated acids should be noted here. These possess the general formulae, $C_nH_{2n-8}O_2$ and $C_nH_{2n-10}O_2$.

in which R_1 and R_2 represent the fatty acyl radicles. Both saturated and unsaturated fatty acids have been isolated from the lecithin molecule. The saturated acids are stearic and palmitic, and unsaturated, oleic, linoleic and linolenic, containing one, two and three double bonds respectively in the molecule, as well as an acid with four double bonds, arachidonic acid ($C_{20}H_{32}O_2$). In lecithin, isolated from plant sources, there is a higher percentage of unsaturated acids than is the case with lecithin from animal tissues, but the saturated acids stearic and palmitic are always present as well.

Kephalin.—On hydrolysis the same products are obtained from kephalin as from lecithin, with the exception that choline is replaced by the weaker base aminoethyl alcohol, $CH_2OH—CH_2NH_2$.

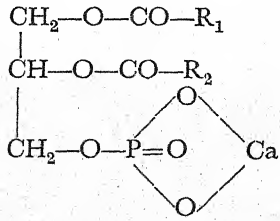
The same fatty acids have been isolated from kephalin as from lecithin with the exception that the only saturated fatty acid that has been found is stearic acid.

Sphingomyelin.—On hydrolysis sphingomyelin yields phosphoric acid, two bases, choline and sphingosine, and fatty acids in equivalent proportions. The base sphingosine is considered to possess the following structure:

$$CH_3 . (CH_2)_{12} . CH{=}CH . CHOH . CHOH . CH_2NH_2.$$

Sphinogosine and therefore sphingomyelin has only been found in animal tissues and will not be considered further here.

Nitrogen-free Phosphatide.—This product isolated from dried cabbage leaves by Chibnall and Channon,* and called by them phosphatidic acid, contains no nitrogen, and has been shown by these investigators to possess the following structure:



where R_1 and R_2 represent the fatty acyl radicles, one of which at least is unsaturated. The presence of calcium in the molecule should be noted. The phosphatide is soluble in ether and probably represents the first recorded instance of an ether-soluble calcium

* *Biochem. J.*, 1927, **21**, 225, 233, 479, 1112.

derivative isolated from a living organism. It has also been isolated from runner-bean leaves and occurs in wheat germ as the magnesium salt.

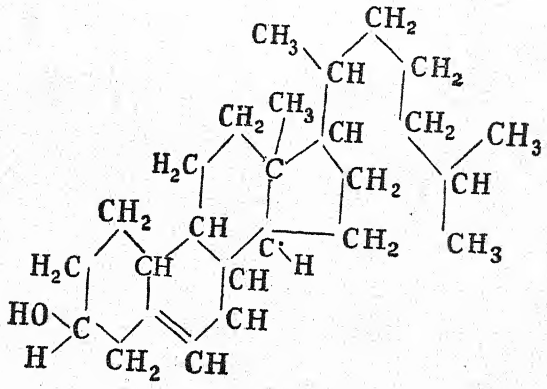
THE WAXES

The waxes are fatty acid esters of monohydric alcohols. They are hydrolysed by boiling alkali in the same way as the true fats, but the time of hydrolysis is more prolonged. The waxes do not normally occur enclosed within cellular tissue. The vegetable waxes exist mainly in the free form on the surface of plants.

UNSAPONIFIABLE RESIDUE OF OILS AND FATS

The term "unsaponifiable residue" is used to cover those substances which are incapable of undergoing further hydrolysis under the conditions used for fat hydrolysis. This does not mean that they are not in ester union with fatty acids prior to hydrolysis which is necessary for their isolation from fats. The sterols are the most important or at any rate most prominent constituents of the unsaponifiable residue of oils and fats.

Sterols.—Of the animal sterols cholesterol has been much investigated. It is an unsaturated monohydric alcohol with the formula, $C_{27}H_{45}OH$. It has been assigned the following constitution:



i.e. it is an unsaturated hydroaromatic compound. In plant tissues, the phyto-sterols have been isolated, and it has been found that these are closely related to cholesterol. An isomer of cholesterol, sitosterol, has been isolated from rye, wheat and linseed

oil. Ergosterol, which occurs in ergot and other fungi, and in traces along with cholesterol in animal tissues, is more unsaturated than cholesterol; it contains three double bonds in the molecule and has the formula, $C_{27}H_{41}OH$. When ergosterol is submitted to ultra-violet radiation, vitamin D is formed.

ANALYTICAL METHODS

Extraction.—The extraction of lipides from tissues for physiological purposes presents a number of difficulties. If the lipides contain unsaturated fatty acids, they cannot be dried at high temperatures without bringing about alteration in the fatty acids through oxidation. If the lipides are to be extracted by means of fat solvents, such as ether, light petroleum, chloroform or benzene, the tissues must first be dried, and this drying must be carried out with care, by using low temperatures, or in an atmosphere of some neutral gas such as nitrogen or carbon dioxide.

Another method that has been much used for animal tissues is first to heat the material with strong alkali in alcoholic solutions so that all forms of fats are hydrolysed, the solution is then acidified and shaken with an exact amount of light petroleum, and aliquots of the light petroleum are removed, and evaporated off under carbon dioxide and the residue weighed. For the full experimental details see Leathes and Raper, *The Fats.*

There are certain chemical constants which are always estimated to identify a specimen of fat, such as the acid value, saponification value, iodine value, thiocyanogen value, Reichert-Meissel value, acetyl value, and percentage of saturated and unsaturated fatty acids. For the practical details of determining these various values, such works as Fryer and Weston, *Oils, Fats and Waxes,* and Jamieson, *Vegetable Oils and Fats,* should be consulted.

Acid Value.—This represents the number of milligrammes of potassium hydroxide required to neutralize one gram of fat.

Saponification Value.—The saponification value of a fat is the number of milligrammes of potassium hydroxide required to saponify one gram of fat.

Iodine Value.—The iodine value of fats or fatty acids is the amount of halogen, reckoned as iodine, taken up by the unsaturated fatty acids present, and expressed as a percentage of the weight of the fat. Thus oleic acid possesses the molecular

weight 282, and since one double bond is present, it can take up two atoms of iodine (2×127), or 90·07 per cent of iodine, and its iodine value is therefore 90·07.

Thiocyanogen Value.—This is an important new constant for fats. It has been found that thiocyanogen (SCN) adds to the double bonds present in unsaturated fatty acids in a different way from bromine and iodine. Thus, while either bromine or iodine will add to all the double bonds in the fatty acid series, thiocyanogen adds to the single double bond of oleic acid, but to one only of the two double bonds present in the linoleic acid, and to two only of the three double bonds present in linolenic acid. If the percentage of saturated and unsaturated acids be known, as well as the iodine and thiocyanogen values, it is possible to calculate the proportion of oleic, linoleic and linolenic acids in a mixture of fatty acids by means of the following equations:

$$O = (100 - G) - 1 \cdot 104 \text{ (Iodine value} - \text{Thiocyanogen value)}$$
$$L = (100 - G) - 1 \cdot 104 \text{ (2 Thiocyanogen value} - \text{Iodine value)}$$
$$L_n = (100 - G) + 1 \cdot 104 \text{ (Thiocyanogen value)}$$

where O represent oleic acid, L linoleic acid, and L_n linolenic acid, and G the percentage of saturated fatty acids in the mixture.

To obtain the percentage of saturated and unsaturated fatty acids, it is necessary to convert the free acids into their lead salts. The lead salts of the unsaturated acids are soluble in ether, whereas those of the saturated acids are relatively insoluble. In this way it is possible to separate the mixture of acids by heating with ether. The method has been made quantitative, and it is usual to use ethyl alcohol in place of ether to effect the separation, under stringent conditions of concentration of mixed acids, time of heating and cooling and temperature of cooling. The residue of the lead salts of the saturated acids is decomposed with hydrochloric acid, and the free acids taken up with ether. The ether is evaporated off, and the residue, which represents the saturated fatty acid fraction of the mixture, can be weighed and the percentage calculated. The percentage of unsaturated acids is obtained by difference.

Acetyl Value.—The acetyl value is a measure of the amounts of hydroxyl groups which a fat contains. The fat is first acetylated by heating with acetic anhydride and the acetylated product is then hydrolysed with alcoholic potash. The acetyl value is

expressed as the number of milligrams of potassium hydroxide required to neutralize the acetic acid that is set free from one gram of the acetylated product when it is saponified.

Reichert-Meissel Value.—This test represents a measure of the volatile acids contained in an oil or fat and is given by the number of cubic centimetres of 0·1N sodium hydroxide solution required to neutralize the liquid fatty acids distilled off from 5 grams of fat.

Hehner Value.—The percentage of fatty acids insoluble in water after saponification of a fat is called the Hehner Value.

FAT METABOLISM

The origin of lipides in different plant organs is one of the first problems to be considered here. It has already been stated that the lipides occur in seeds, spores and fruits, where they obviously function as reserve foods, but lipides are also present in leaves. Their function and origin in leaves is not properly understood, although some recent investigations which will be considered have shed a certain amount of light on the question.

It was at one period considered that fats were formed in photosynthesis, and the alga Vaucheria was given as the example. There is, however, at present, a good deal of doubt as to the chemical nature of the fat-like substance present in Vaucheria. Two other sources have been claimed for the origin of fats in plants and animals, namely, proteins and carbohydrates. With regard to the proteins, the evidence available is from the animal side of physiology. A great deal of this work has since been shown to be erroneous, and the only authentic case of fat formation from protein in the animal is the case of the larvae of the fly Calliphora. The evidence that fats are formed from carbohydrates is strong. The experiments of Gilbert and Lawes at Rothamsted on the feeding of pigs, sheep and oxen, and also later investigations, placed the matter beyond all reasonable doubt that fats are formed from carbohydrates in the animal economy.

In plants, the place of synthesis of fats and the precursors of fats have been the main objective. Unfortunately the experimental technique employed for this purpose was by no means suitable. In the older investigations, e.g. Rousille in 1878, and Funaro in 1880, the total ether extracts were looked upon as representing the amount of fats present, but the total ether extract will also

contain other organic substances besides fats, such as resins, higher alcohols and hydrocarbons. The replacement of ether by light petroleum is in many ways to be preferred in this connection, but even so, other bodies than fats will be extracted at the same time, although the number will be more limited than with ether.

The reserve fats in seeds and fruits seem to be manufactured *in situ* and not translocated from other parts of the plant. The work of Le Clerc du Sablon has shown that in certain nuts, e.g. almond and walnut, that with decrease of carbohydrate during the ripening process there is an increase in fat. Some of his data are given below:

Walnut

(*Percentage*)

Date of Collection	*Oil*	*Glucose*	*Sucrose*
July 6	3	7·6	0·0
Aug. 1	16	2·4	0·5
Aug. 15	49	—	0·6
Sept. 1	52	—	0·8
Oct. 4	62	—	1·6

Almond

(*Percentage*)

Date of Collection	*Oil*	*Glucose*	*Sucrose*	*Starch*
June 9	2	6·0	6·7	21·6
July 4	10	4·2	4·9	14·1
Aug. 1	37	—	2·8	6·2
Sept. 1	44	—	2·6	5·4
Oct. 4	46	—	2·5	5·3

The nature of the fats formed during the ripening process of seeds and fruits has not been investigated with any great thoroughness. The inherent difficulties of such an investigation are very great, but nevertheless it is a pity that more has not been done in this direction, as a considerable amount of light might be cast on the actual stages of the various reactions concerned in fat formation in plants. According to Ivanov, there is an increase in

the iodine value of the oil in linseed during ripening. It was found that during a period of seven weeks the iodine value rose from 120·6 to 175·3. This, however, may be a special case, for there is no increase in the iodine value of oil from poppy seed, colza and hempseed during ripening.

The formation of fat in Saccharomyces has been investigated by McLean and Hoffert,* who found that as the reserve carbohydrates disappeared, the fats in yeast increased in the presence of oxygen. They also found that such substances as acetone, glycerol, and the sodium salts of formic, propionic and butyric acid could bring about fat formation in yeast. On the other hand, various alcohols, such as butyl, propyl and isoamyl alcohol did not lead to fat formation. The sugars, glucose, fructose and sucrose gave rise to fat, whereas maltose led to the formation of reserve carbohydrate and was less efficient in fat formation than the three sugars mentioned above. According to Stevenson and Whetham,† the Timothy grass bacillus can synthesize fat from sugar and when the supply of carbohydrate is exhausted the fat rapidly disappears. There is a change in the respiratory quotient which suggests the fats are being used in respiration. On the balance of evidence at present available, it would appear that the precursors of fats in plants as in animals are carbohydrates.

As far as the actual chemical reactions in the formation of fats in the living organism are concerned, it is clear that the process must take place in stages. In the first place, there is the formation of the fatty acids to be considered, and in the second place, the synthesis of the triglyceride by union between glycerol and fatty acids. This second reaction is undoubtedly brought about under the agency of the enzyme lipase. We have already seen (see Chapter VII) that lipase is able to accelerate either the hydrolysis of fats to glycerol and free fatty acids, or the reverse reaction of synthesis of fats from free fatty acids and glycerol. The question of the sequel of events concerned in the synthesis of the fatty acids is more speculative.

In the first place, it has been pointed out that the fatty acids which have been isolated from living organisms contain an even number of carbon acids. This fact suggests that the building up of a molecule of fatty acid in the living cell is brought about by the union of two carbon atoms at a time. A further fact that

* *Biochem. J.*, 1923, **17**, 720.
† *Proc. Roy. Soc.* (*Lond.*), 1922, **93B**, 262; 1924, **95B**, 200.

must be considered in this connection is that the acids that have been isolated from living organisms are both saturated and unsaturated. It follows, therefore, that the steps of synthesis must be of such a nature that slight changes in the conditions will give rise to either saturated or unsaturated acids.

The presence of acids with eighteen carbon atoms in the molecule which appear over and over again in plant and animal tissues, e.g. stearic acid, $C_{18}H_{36}O_2$, oleic acid, $C_{18}H_{34}O_2$, linoleic acid, $C_{18}H_{32}O_2$, and linolenic acid, $C_{18}H_{30}O_2$, led Emil Fischer to the view that their synthesis might be brought about by the condensation of three molecules of hexoses, followed by oxidation and reduction. There is a great deal of evidence against such a view. In the first place, there is no reason why a pentose and hexose should not combine in this way, which would lead to the formation of a substance with an uneven number of carbon atoms; and secondly, butter fat contains fatty acids with an even number of carbon atoms in the molecule, which vary from four to twenty, and these fatty acids could be formed from hexoses, pentoses or trioses.

The question arises therefore, What is the nature of the intermediate substance between carbohydrates and fatty acids? It has already been stated that the process of building up fatty acids in the living cell most probably takes place by the addition of two atoms of carbon at a time, or by the union of several molecules of some substance containing two carbon atoms. One probability that presents itself in this connection is acetaldehyde, $CH_3 . CH : O$. It was suggested in 1878 by Nencki that in the formation of butyric acid from lactic acid during fermentation, acetaldehyde was an intermediate stage. The acetaldehyde thus formed then condensed with a second molecule of aldehyde, the so-called aldol condensation, and the resulting aldol by molecular rearrangement gave rise to butyric acid:

$$(1)\quad \underset{\text{Lactic acid}}{CH_3CHOH . COOH} = \underset{\text{Acetaldehyde}}{CH_3CH : O} + CO_2 + H_2$$

$$(2)\quad CH_3CH : O + CH_3CH : O = CH_3CHOHCH_2CH : O$$

$$(3)\quad \underset{H . H}{CH_3CHOHCH_2CH} : \underset{O}{O} = \underset{\text{Butyric acid}}{CH_3CH_2CH_2COOH} + H_2O$$

This scheme of aldol condensations for the building up of the

higher fatty acids was further developed by Magnus-Levy* and also by Leathes.† As Leathes has pointed out, such a reaction as the formation of butyric acid from aldol is not known *in vitro*. It is well known that aliphatic hydroxy compounds are difficult to reduce. An alternative suggestion to that given above is that the aldol loses a molecule of water to give crotonaldehyde, and that this unsaturated aldehyde is then reduced by the hydrogen formed in the initial stage of the reaction to butaldehyde, and the last stage of the reaction is the oxidation of the butaldehyde to butyric acid by oxygen from the air. The objection that aliphatic aldehydes condense with acetaldehyde to give compounds with branched chains and never with straight chains of carbon atoms, does not apply here, for it has been shown by Raper that when acetaldehyde condenses with itself to give aldol the latter undergoes auto-condensation to give a straight chained derivative.

Smedley and Lubrzynska‡ have further extended this suggestion and have considered that pyruvic acid is first formed from carbohydrate and forms the initial point for fatty acid synthesis in the cell. The pyruvic acid is oxidized by the enzyme carboxylase (see Chapter XIII) to acetaldehyde by elimination of carbon dioxide from the molecule, and the acetaldehyde thus formed condenses with a further molecule of pyruvic acid to give an unsaturated ketonic acid:

(1) $\underset{\text{Pyruvic acid}}{CH_3CO\,.\,COOH} = CH_3CH : O + CO_2$

(2) $CH_3CH : O + CH_3CO\,.\,COOH$
$= CH_3CH : CH\,.\,CO\,.\,COOH + CO_2$

The ketonic acid thus formed may react in one of two ways: it may lose a molecule of carbon dioxide to give crotonaldehyde, or it may undergo oxidation to give crotonic acid. If the first reaction takes place, then the crotonaldehyde so formed may condense with a further molecule of pyruvic acid to give a ketonic acid once more, this ketonic acid may either lose a molecule of carbon dioxide to give an unsaturated aldehyde or it may undergo oxidation and reduction to give a saturated acid. The formation of caproic acid by this scheme is given on p. 266.

* *Engelmann's Archiv.*, 1902, 365.
† *Problems in Animal Metabolism.*
‡ *Biochem. J.*, 1913, 7, 364.

$$CH_3CH : CH . CO . COOH = CH_3CH : CH . CH : O + CO_2$$

$$CH_3CH : CH . CH : O + CH_3CO . COOH$$
$$= CH_3CH : CH . CH : CH . CO . COOH + H_2O$$

$$CH_3CH : CH . CH : CH . CO . COOH$$
$$= CH_3CH : CH . CH : CH . CH : O + CO_2$$

$$2\ CH_3CH : CH . CH : CH . CH : O + 4H_2 + O_2$$
$$= 2CH_3 . CH_2 . CH_2 . CH_2CH_2 . COOH$$

Caproic acid

The main difficulty in accepting this theory is the present uncertain position of pyruvic acid as an intermediate metabolite in carbohydrate decomposition in the animal. The difficulty does not arise as far as the yeasts are concerned, for it has been shown by Neuberg and his co-workers that this acid occurs as an intermediate product in alcoholic fermentation.

With regard to the formation of glycerol, this substance is closely related to glucose chemically, and may find its origin from this source. Further, glycerol is one of the products formed in alcoholic fermentation by yeast. The reactions concerned in the formation of glycerol from glucose in alcoholic fermentation are discussed in detail in Chapter XIII.

The fate of the lipides stored as reserve food in storage organs can be conveniently considered here. It has been seen that the whole of the evidence at present available points to the carbohydrates as being the precursors of the lipides stored in seeds and fruits. The question now arises as to the behaviour of the lipides at germination.

It has been shown by Le Clerc du Sablon and also by Miller* that in the early stages of germination of seeds with a high fat-content, there is little diminution in amount. In the almond it was found by du Sablon that the percentage of fat fell from 50 per cent to 45 per cent when the roots had reached a length of 2 cms. During the subsequent stages of development, however, the amount of fat fell rapidly, and when the root had attained a length of 20 cms., only 7 per cent of fat was found to be present in the seedling. Miller, who used the sunflower, *Helianthus annuus*, obtained very similar results. Initially the seed was found to contain 56 per cent of fat, and this value fell to 51·9 per cent when the cotyledons had reached above ground. Thereafter, the amount of fat fell rapidly, and when the cotyledons had com-

* *Ann. Bot.*, 1910, **24**, 693; 1912, **26**, 889.

pletely expanded, the amount of fat had fallen to 13·5 per cent.

During germination considerable changes occur in the composition of the neutral fat. With time there is an increase in the amount of free fatty acids, brought about by the hydrolytic activity of lipase. The following figures taken from du Sablon for linseed illustrate this point:

Length of Root in Centimetres	*Per cent Total Oil*	*$Ba(OH)_2$ required to neutralize 100 Grams Oil in Cubic Centimetres*
0·0	37·9	0·3
0·6	36·4	0·3
1·2	30·1	0·9
2·2	22·1	3·9
3·2	17·5	4·1
4·0	11·0	11·4
4·2	9·3	17·7

The greatest amount of hydrolysis is found while there is a rapid fall in the amount of fat. Miller also found there was an increase in the acid value of the fat from *Helianthus annuus* during germination. In the resting seed the acid value was found to be 1·6, in seedlings seven days old, the value had increased to 4·6 and the amount of fat present had fallen to 33 per cent of that originally present, while, when the fat-content had diminished to 5·3 per cent, the acid value had risen to 66·8.

During germination there is a fall in the value of the iodine number. In *Helianthus annuus*, Miller found the initial iodine value to be 125, and this value fell to 120 when the seedlings were seven days old, and there was a further fall to 111·8 after fourteen days. According to Ivanov, this result is due to the fact that the unsaturated acids are used up first in germination, and as a result the iodine value falls. Miller has advanced an elaborate view that the fall in the iodine value is brought about by the formation of hydroxylated acids or saturated acids of lower molecular weight from the unsaturated acids. The evidence which he advances for this view is extremely slender. He found a small decrease in the acetyl value of the ether extract of the cotyledons, but a well-marked increase in the acetyl value of the ether extract from roots and hypocotyl; and he considers this important evidence that hydroxylation of fatty acids has occurred.

As he extracted his material after drying with ether, it is difficult to say what other products besides fats were present. Moreover, it has been shown by von Fürth* that the acetyl value of sunflower seedlings falls during germination. Thus, in the resting seed, the fat was found to have an acetyl value of 87·5 and this fell to 50·5 in the seedling.

The question as to whether fat is converted into carbohydrates in the animal economy is still a matter of considerable controversy, but the balance of evidence seems to be against such a view. In the plant, on the other hand, the available evidence points very strongly to conversion of reserve fats in seeds to carbohydrates, and this is what normally happens when fatty seeds germinate. The process, fundamentally considered, is one of oxidation, and shows the great difference between the oxidation of fat in animals and plants. In the animal, fat is completely oxidized to carbon dioxide and water, but in such plant organs as germinating fatty seeds with high fat-content, the process stops short of this stage of completeness, and part of the intermediate products, sugars, are used for other constructional purposes such as building up of new tissues.

In 1833 it was observed by de Saussure that the volume of oxygen absorbed by germinating fatty seeds was greater than the volume of carbon dioxide evolved, and many years later (1882) Godlewski found that the respiratory quotient (CO_2/O_2) for the period of most rapid absorption of oxygen during the germinating process varied between 0·55 and 0·65 and suggested the following series of reactions in the case of the conversion of triolein to starch:

$$(C_{18}H_{33}O_2)_3C_3H_5 + 3H_2O = 3C_{18}H_{34}O_2 + C_3H_8O_3$$
$$C_{18}H_{34}O_2 + 17O_2 = C_6H_{10}O_5 + C_2H_8O_3 + 10CO_2 + 8H_2O$$

After preliminary hydrolysis of the glyceride to oleic acid and glycerol, the fatty acid was oxidized to starch, some hypothetical substance ($C_2H_8O_3$), carbon dioxide and water. The respiratory quotient for such a reaction would be $\frac{10CO_2}{17O_2} = 0{\cdot}59$. These reactions show the formation of 58·3 grams of starch for every 100 grams of fat consumed. Detmer obtained for hemp seed 8·64 per cent of starch for 15·56 per cent of loss of fat from

* *Beihr. chem. Physiol. Path.*, 1904, **4**, 430.

the seeds during the first seven days of germination. On Godlewski's equations, the values should be 9·07 grams of starch from 15·56 grams of fat consumed. This result is in very fair agreement, and shows that some such transformation is possible.

There are many observations available to show that the reserve lipides of seeds are converted into carbohydrates. It was found by du Sablon, and also by Green and Jackson, that sucrose is first formed as fat disappears during the course of germination. Miller showed that the amount of sugar in the resting seed of sunflower was 4·1 per cent, and beyond a trace of reducing sugar, this was entirely composed of sucrose. In the early stages of germination, the amount of sugar fell rapidly and then slowly increased in amount until the cotyledons unfolded. During the whole of this period only sucrose was found to be present, but once the cotyledons had unfolded, reducing sugars made their appearance and, in seedlings that were ten days old, were the only sugars found to be present. He also showed that the increase in carbohydrate during germination of the sunflower could not be due to the reserve protein in the seeds, and that the fats were the only other possible source.

Some further observations on this matter have been made by Murlin* and his co-workers, using castor bean seeds. It was found that the respiratory quotient for the seeds varied between 0·3 to 0·55, while in young seedlings the value for the cotyledons and hypocotyl rose to 0·78 to 1·0. If carbohydrate is being entirely used in respiration, the quotient will be unity, whereas if fat is being used, the quotient will be less than unity. From the values obtained for the cotyledons and hypocotyl, it is evident that there is considerable combustion of carbohydrate.

It was discovered that the seat of fat transformation into carbohydrate was in the endosperm of the seed, and with increase in the stage of germination there was an increase in the respiratory quotient indicating a change from a substance poor in oxygen to a substance rich in oxygen. Analyses were also made of seeds and seedlings for water, ash, ether extract, protein, crude fibre, total reducing matter as invert sugar and later glucose. The values obtained are given on p. 270.

It will be seen from these figures that as the fat-content decreases (ether-soluble fraction), sugars increased. Estimations were made until the hypocotyls were 250 mm. in length, and

* *J. Gen. Physiol.*, 1933, **17**, 283, 303, 311; *J. Nutrition*, 1933, **6**, 523.

this decrease in fat and increase in sugars was found to continue. It seems reasonably certain from these figures, that there is conversion of fat into carbohydrate in the germinating fatty seed. The other fractions determined, such as ash and protein, show a relatively constant value throughout the whole experimental period. The marked changes are increase in sugar and to a lesser degree increase in fibre with decrease in fat.

An attempt was made, with no very marked success, to draw up a carbon balance sheet, but Murlin considers that his figures support the view that for every six molecules of ricinoleic acid consumed in germination, two are converted into sucrose and one

Chemical Composition of Germinating Castor Bean Seeds

(*Percentage*)

Length of Hypocotyl Millimetres	*Ether Extract*	*Total Reducing Matter as Invert Sugar*	*Glucose*	*Crude Fibre*	*Protein*	*Ash*
Ungerminated	67·85	1·18	—	1·87	25·72	2·61
5–10	63·74	1·62	—	2·62	26·32	2·63
10–20	61·55	6·02	—	3·23	24·26	2·57
20–35	57·40	6·88	—	2·37	25·86	2·48
35–45	45·30	15·68	—	4·02	26·51	2·77
45–60	38·03	17·26	—	7·01	27·48	2·43
60–80	32·58	25·84	4·47	4·08	26·84	2·86
80–100	25·28	26·63	6·32	5·01	27·26	3·04

into cellulose, while three are oxidized to carbon dioxide and water.

The problem of the translocation of fats must be discussed here. There are two views to be considered. In the first place, are the fats translocated as fats from one part of the plant to another, or are they first converted into some other product, e.g. carbohydrates, translocated in this form, and then resynthesized again to fats? Sachs maintained that fats were translocated as fats. Schmidt, on the other hand, claimed that free fatty acid and not neutral fat is the mobile form of lipide in the plant. Rhine* has made a reinvestigation of this problem and measured the respiratory quotient for various fatty seeds, such as sunflower

* *Bot. Gaz.*, 1926, **82**, 154.

and cotton, as well as for starchy seeds; for example, pea, wheat and barley.

It was found by Rhine that the respiratory quotient for fatty seeds during germination was 0·770 and for starchy seeds 0·775, i.e. the values were the same in the two classes. It is evident, therefore, that as far as the fatty seeds are concerned, the food supplied to the hypocotyls from the storage regions arrives there in a form giving the same respiratory quotient as starchy seeds. Presumably in starchy seeds the carbohydrate is moving in the form of soluble sugars, and it is evident that fat cannot be moving into the hypocotyls as fat in the case of the fatty seeds, or the respiratory quotient would be different. Moreover, Rhine was unable to find any gradient of fat from the seed to the cotyledon. Actually, a gradient was found from cotyledon to the base of the hypocotyl:

Percentage Ether Extract in Parts of Fatty Hypocotyls

(*Results Expressed as Percentage Ether Extract on Wet Weight*)

Seedling	*Tips*	*Middles*	*Bases*
Cotton	5·75	2·24	1·33
Sunflower	5·31	1·34	0·66
Squash	2·59	0·60	0·22

Rhine is of the opinion that in fatty seeds fats are not translocated as such, nor are they translocated as the free fatty acids, but that the fat first undergoes hydrolysis and is then converted into sugar and removed as such into the developing regions.

The function of the plant phosphatides has recently been tentatively investigated by Chibnall* and others. In the cabbage leaf neither lecithin nor kephalin are to be found, but the nitrogen-free phosphatide, phosphatidic acid, is present as the calcium salt. It would therefore seem that the synthesis of phosphatides in the leaf stops at the diglyceride-phosphoric acid stage. Combination with either choline to give lecithin or aminoethyl alcohol to give kephalin does not appear to take place in the green leaf. Both lecithin and kephalin are esters, whereas calcium phosphatidate is a true salt of calcium. Calcium as well as magnesium phos-

* *Biochem. J.*, 1927, **21**, 225, 233, 479, 1112.

phatidate, is soluble in ether but insoluble in water, so that neither of these salts can exist in the aqueous phase of the cell. On the other hand, the sodium and potassium salts are soluble in water, but insoluble in ether, and if these be present in the cell they will be present in the aqueous phase.

It is a well-known fact that in animal tissues the lipides can be divided into two fractions. One of these fractions varies in amount (*élément variable*), and represents the true reserve fat, whereas the other fraction cannot be diminished in amount without bringing about death (*élément constant*). This *élément constant* forms an essential component of the protoplasm. In the animal economy, the *élément variable* is chiefly composed of relatively saturated fatty acids, such as occur in the adipose tissues, while the *élément constant* is composed of relatively unsaturated fatty acids which are to be found in the heart, kidneys and liver and contain much phosphatide.

The lipides of fatty seeds contain a relatively large amount of saturated fatty acids and are considered by Chibnall to represent possibly the *élément variable* of the animal physiologist. The statement that the lipides of seeds contain relatively saturated fatty acids is somewhat stretching a point; in wheat germ, for example, from 84 to 85 per cent of the fatty acids are unsaturated, the main unsaturated acid being linoleic acid. Nevertheless, leaving this criticism aside, the lipides of seeds certainly represent reserve food and are used in germination. Such being the case, are the lipides of the leaf, for example, to be considered as representing the *élément constant*? In other words, are they to be considered as components of the leaf cytoplasm and will they persist after death from inanition? Chibnall is inclined to this view and has produced a certain amount of supporting evidence.

Chibnall and Sahai* have examined the problem of the function of phosphatides in the leaf of the brussels sprout (*Brassica oleracea* var. *bullata*). If calcium phosphatidate is playing the same part in the leaf as lecithin and kephalin in the metabolism of the animal it is to be expected that the glycerides would disappear, and the calcium phosphatidate remain on starvation. If, however, it is concerned in the mobilization and translocation of lipide material, it should disappear on starvation since the sodium and potassium salts are soluble in water.

Cut leaves of brussels sprouts were kept in the dark with their

* *Ann. Bot.*, 1931, **45**, 489.

petioles under water and no change was discovered in the amount of phosphatidate at the end of this starvation period. One curious change was discovered; the sugars of the lamina increasing instead of decreasing in amount. This result was found to be due to the lamina drawing on sugar from the fleshy petioles. The results obtained from this investigation were not altogether satisfactory, and a more extensive investigation of the question was made by Jordan and Chibnall* on the runner bean (*Phaseolus multiflorus*).

The seeds of the runner bean were found to contain the phosphatides, lecithin and kephalin. The phosphatide fractions of the cotyledons, embryo axes, young germinating plants, prophylls and pinnate leaves were investigated, and phosphatidic acid was found to be present as the magnesium salt in small amount in the cotyledons and embryo axes. In the early stages of germination, magnesium phosphatidate was found to increase greatly in amount, but the calcium salt did not make its appearance until the prophylls had expanded, after which magnesium was rapidly replaced by calcium. Lecithin and kephalin were found to persist in small amounts throughout. The fact that phosphatides disappeared at germination more rapidly than they reappeared in the growing organs, suggests that the phosphatides in the resting seeds must function chiefly as reserve food. When, however, the leaves were placed under starvation conditions in the dark, protein and phosphatide disappeared slowly and together, whereas the glyceride fatty acids disappeared fairly rapidly, suggesting that the latter function as reserve food. The fact that phosphatides and proteins only disappear slowly and together under starvation conditions supports the view that the leaf phosphatides form an integral part of the protoplasm and are of the nature of the *élément constant* of animal tissues.

THE FUNCTION OF LIPIDES IN THE LIFE OF THE CELL

We have already seen that fats play an important part as reserve foods in the animal and plant economy, and until recent times the fats were looked upon as mere stores of potential energy. There can be no doubt that on this score alone, they are of the utmost importance to the living organism, but other and equally important functions are to be ascribed to the lipides of the cell. The question of the *élément variable* and the *élément constant* of the

* *Ann. Bot.*, 1933, **47**, 163.

cell has been discussed in the last section. The necessity for lipide material in the cell as long as it is alive, is a pointer that lipides play some intimate part in cellular reactions, other than acting in the capacity of mere reserve food material. It has already been shown that this essential lipide material (*élément constant*) of the living cell is in the form of phosphatides and cholesterol.

The importance of the lipides in cellular activities has not been sufficiently realized heretofore, largely because the usual definition of a fat as a triglyceride is not sufficiently broad. The physiological importance of the lipides lies in the properties of the long chains of carbon atoms composing the molecules of the component fatty acids, and the physiology of these fatty acids rests upon the physical and chemical properties of these carbon chains and cannot be merely confined to their behaviour when they are only combined with glycerol alone as simple glycerides.

Many of the fatty acids can only fulfil their physiological destiny by entering into combination, even if it only be a temporary combination, with other substances than glycerol. For this reason alone, it would be of greater interest had the methods that have been adopted to investigate the lipides of the plant cell estimated the fatty acids freed from all combinations into which they may have entered, and the results been given as such.

It has already been shown in the chapter on permeability (Chapter IV) that a number of the phenomena exhibited by cells, such as specific permeability to particular ions and molecules and impermeability to others, appear to point to the presence of some kind of surrounding membrane on the surface of the cell which is in the form of a definite layer. It will be recalled that some of the speculations regarding this membrane have considered it to be of the nature of lipide material containing phosphatides.

The phosphatides, in addition to containing a great structural similarity to the neutral glycerides, also possess one free acidic group attached to the phosphoric acid residue, as well as a basic nitrogen group. They can therefore behave as amphoteric electrolytes, and at their iso-electric point, it might be expected that they would show greater physical instability than at other hydrogen-ion concentrations. At a pH below their iso-electric point they will form salt-like combinations with acids, while at a pH above the iso-electric point they will combine with bases. In this respect the phosphatides may be compared with the

proteins, and lecithin with electrolytes shows many of the precipitation phenomena of proteins. Thus salts with monovalent kations have no effect on an aqueous emulsion of lecithin, whereas di- and trivalent cations bring about precipitation within certain definite concentrations, trivalent cations being the most efficient.

In the plant cell there is present an aqueous solution containing electrolytes, and in such a solution the lipides will form a two-phase system. In the case of the phosphatides, the ionic equilibria at the intersurfaces of such a heterogeneous system will probably be controlled by the Donnan equilibrium. In this way the presence of the phosphatides helps to give a physical explanation for various cellular activities which require differences in ionic concentrations on the two sides of a limiting membrane.

In this regard, the important investigations of Adam* on surface films of fatty acids and similar substances require discussion. Molecules are frequently spoken of as being spherical. This is not correct. Such a substance, for example, as palmitic acid does not possess a spherical molecule. The molecule of palmitic acid, as far as can be told, possesses an elongated structure with different characteristics at either end. A molecule of palmitic acid, or for the matter of that any molecule of the long-chain fatty acids, possesses the same essential structure as a paraffin hydrocarbon, the main difference being that in a paraffin hydrocarbon the groups at either end of the molecule are the same, whereas at one end of the molecule of a fatty acid there is a carboxyl group. The presence of this carboxyl group confers upon the molecule of the fatty acid properties which are not apparent in any other part of the molecule, or in the molecule of a paraffin hydrocarbon.

Adam has developed a technique which is really an extension of earlier experiments of Langmuir and Hardy, of spreading on a surface of water films of fatty acids, esters of fatty acids, and other derivatives containing long hydrocarbon chains. It was found that with the fatty acids the molecules orientated themselves on the water surface in a definite way. A weighed amount of fatty acid was dissolved in benzene, and three or four drops were allowed to fall upon the water surface contained in a special trough. The benzene evaporated off and left a known number of fatty acid molecules in the resulting film, and the area of these molecules was then determined by simple means. It was shown

* *Proc. Roy. Soc.* (*Lond.*), 1922, **101**A, 452, 516.

by Adam that the molecules of fatty acid spread themselves over the water surface as a thin film one molecule thick (monolayer) and exhibit polarity. The fatty acid becomes anchored, so to speak, by its carboxyl group to the surface of the water, while the remainder of the molecule projects upwards. The behaviour of hydrocarbons in this respect is quite different, the molecules tending to stick together, as far as possible out of contact with the water.

The properties of substances when in the form of such films are quite different from those which they exhibit when in bulk. Palmitic acid, for example, which melts at 63° C., remains liquid in such films at room temperatures, and at some 30° below its melting-point it passes into a physical condition resembling that of a gas with its molecules all anchored at one point, or as Leathes* puts it, "a gas in two dimensions instead of three."

Leathes has shown that lecithin forms similar "gaseous" films at 5° C., while if cholesterol, which itself forms a liquid film definitely anchored to the surface of water, be mixed with the lecithin, the molecules of the expanded films of fatty acids and lecithin tend to draw close together.

When a lateral compressing force is applied to these monolayers of fatty acids, they solidify. This solidification is shown by the fact that motes, which move rapidly about on the surface of the film, suddenly become stationary. But even in such a solid film in which the molecules of fatty acid are closely packed together, just as they are when the acid is in bulk, water is still able to pass through freely. This is particularly well shown by lecithin. A lecithin film is anchored to a water surface by means of the large glyceryl cholyl phosphoric acid group of the molecule, and this large group prevents close packing of the fatty acid chains.

The importance of these findings of Adam and Leathes cannot be overestimated. Their importance lies in the fact that what amounts to practically a new physical state of matter at an intersurface has been discovered, and it need scarcely be pointed out that living matter is one mass of intersurfaces. A further important fact about these discoveries is that the matter at these intersurfaces is not arranged in any random manner, but that the molecules assume definite polar arrangements with regard to one another which must necessarily deeply influence their inter-

* *Lancet*, 1925.

actions. In place of random and haphazard mixture there is ordered array and this idea may be extended to the whole of the protoplasm of the cell.

Another important point that has been raised by Leathes may be conveniently considered in this connection. If a streak of solid lecithin be made on a slide and then covered with water and observed under the microscope, complicated outgrowths soon begin to show. These outgrowths are similar in appearance to the mycelium of a fungus, hence the name *myelin* forms. Lecithin also gives these myelin forms in the presence of N/100 ammonia, potassium hydroxide and sodium hydroxide solution, but the presence of N/100 calcium hydroxide solution prevents the outgrowths. This result is apparently due to some special property of the calcium ion, for in the presence of N/100 barium hydroxide solution the outgrowths are freely formed. These outgrowths also take place in the presence of N/100 acetic, hydrochloric and sulphuric acids. Egg albumin in sodium phosphate solution shows very modest outgrowths, while the paraffin hydrocarbons will not spread at all in this way. According to Leathes, the essentials of this phenomena are the same as those concerned in the spread of fatty acids on a water surface. It is the carboxyl group that is responsible for the spread of the fatty acid. In lecithin, however, the situation is more complicated, for there are two additional components, phosphoric acid and choline; and the presence of this large polar group with new polar points gives to the lecithin properties not possessed by the simple fatty acid. This complex polar group is apparently more strongly drawn to the water, and as many molecules as possible crowd up to the water surface. As a result the lecithin presents to the water more and more surface, and so these extraordinary outgrowths result. Film formation takes place in all directions and not only in one plane. Meanwhile, water, by passing in between the molecules without destroying the cohesion of the paraffin chains, is imbibed into the budding outgrowths and apparently brings about still further polarization patterns among the deeper molecules within the outgrowths.

Leathes is of the opinion that these phenomena have an important bearing on the distribution of lipide material in the cell substance. They certainly serve to show how the older ideas of the "lipoid membrane" may be extended in a way more in tune with the true physiological activities of the cell.

CHAPTER X

NITROGEN METABOLISM

THE term nitrogen metabolism is used to cover the various reactions concerned in the synthesis of complex nitrogenous compounds found in the plant from such simple sources as nitrates, ammonium compounds, carbon dioxide and water. The green plant, unlike an animal, is a synthetic machine and is able to build up these very complex substances from the simple initial products indicated above. An animal, on the other hand, must be supplied with its nitrogen already elaborated as protein. The exact steps whereby the green plant is able to synthesize the various complex nitrogen compounds necessary for active metabolism are not properly understood.

The proteins are the most important nitrogen compounds found in living organisms. They are to be found in every cell and form an integral part of the protoplasm. Proteins are complex compounds of high molecular weight, containing carbon, hydrogen, nitrogen and sulphur in the molecule, whilst phosphorus has been found in a few proteins. They are essential to all life processes, and in plants are found in the dissolved state in the cell sap, in the semi-dissolved state in the cytoplasm and in the insoluble condition in storage organs, such as tubers, bulbs, roots, buds and seeds.

The insoluble protein found in storage organs may be either in the crystalline or amorphous state. Protein has been found in the crystalline condition in the seeds of cotton, sunflower, flax and the potato tuber. The only plant proteins that have been at all completely investigated are those of various seeds, since they occur in these organs in relatively large amounts and are more readily isolated than from other plant structures.

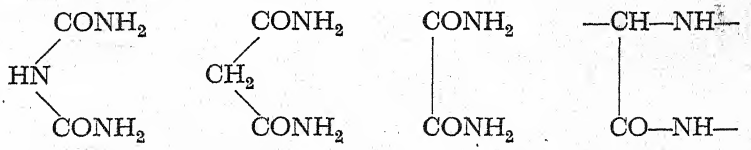
Proteins are compounds of high molecular weight and are colloidal in nature, belonging to the class of emulsoid colloids. In solution they do not pass through parchment or collodion membranes. Their solutions are optically active and rotate the plane of polarized light to the left. Solutions of proteins coagulate on keeping, and the pH of the medium is an important factor in this process of coagulation. A low pH favours coagulation, whereas if the pH of the solution be high coagulation may be

prevented. The addition of various salts brings about precipitation of proteins from their solutions. No permanent chemical change is produced when they are precipitated by the addition of inorganic salts, and they still retain their original properties and solubilities. One group of proteins, the peptones, are not precipitated by the addition of salts.

The figures given below represent the average composition of plant proteins: Carbon = 50·6–55·0 per cent; Hydrogen = 6·5–7·3 per cent; Oxygen = 21·5–23·5 per cent; Nitrogen = 15·0–19·3 per cent; Sulphur = 0·3–2·2 per cent; Phosphorus = 0·0–0·9 per cent.

Qualitative Reactions of Proteins.—The proteins give certain characteristic colour reactions with different reagents, owing to the presence of the same groupings in the molecules of different members of the group.

(*a*) *Biuret Reaction.*—On the addition of a solution of sodium hydroxide to a protein, followed by the addition of a dilute solution of copper sulphate, drop by drop, a reddish-violet colour is developed. This reaction is called the biuret test, because this reaction is given by biuret itself, as well as compounds containing the following groupings in the molecule:



Biuret Malonamide Oxamide Grouping in protein molecule

Millon's Reaction.—When a solution of protein or suspension of protein in water is boiled with Millon's reagent, a pink colour is developed. Millon's reagent is prepared by adding one part of mercury to two parts of concentrated nitric acid in the cold. When the mercury has entirely dissolved, and for complete solution a little gentle warming may be necessary, the solution is diluted with two volumes of water. The colour reaction depends on the presence of the amino-acid tyrosine (see below) in the protein molecule. If the protein does not contain tyrosine the reaction will not be given.

Xanthoproteic Reaction.—If the protein is heated with concentrated nitric acid, a yellow colour is developed. On the addition of ammonia the colour changes to orange-red.

Hopkin's-Cole Reaction.—A solution of protein is first treated with an equal volume of "reduced oxalic acid," prepared by the action of sodium amalgam on oxalic acid. This "reduced oxalic acid" contains the substance glyoxylic acid (CH : O . COOH). Concentrated sulphuric acid is now carefully added. A purple ring forms at the junction of the two liquids. This reaction is dependent upon the presence of the amino-acid tryptophane in the protein molecule.

Molisch Reaction.—The addition of two drops of an alcoholic solution of α-naphthol and one to two volumes of concentrated sulphuric acid to a solution of a protein gives a violet colouration. This reaction is only given by proteins containing a carbohydrate group in the molecule.

Sulphur Reaction.—The solution of protein is boiled with a few drops of lead acetate solution in the presence of excess of sodium hydroxide. A black precipitate of lead sulphide is formed.

Proteins are precipitated from solution by such substances as trichloracetic acid, potassium ferrocyanide, tannic and picric acids, as well as the salts of the heavy metals, e.g. lead acetate, mercuric chloride and ferric chloride.

The Iso-electric Point of Proteins.—Proteins are amphoteric electrolytes and are able to behave as either acids or bases. In the presence of acids they function as bases:

$$R—OH \rightleftharpoons R^{+} + OH'$$

whereas in the presence of bases they function as acids:

$$R—OH \rightleftharpoons RO' + H^{+}$$

This phenomenon is called *electrolytic tautomerism.* It is evident that at some particular concentration of hydrogen-ions, the number of protein ions and cations will be exactly equal, the protein will not move to either anode or cathode, and its solubility at this particular concentration of hydrogen-ions will be at a minimum. The particular concentration of hydrogen-ions required to bring about this result is the iso-electric point of the protein (see Chapter II).

The iso-electric point of various plant proteins has been determined by Pearsall and Ewing,* and some of their values are given on p. 281.

* *Biochem. J.*, 1924, **18**, 2; *New Phyt.*, 1924 **23**, 193.

Edestin pH	= 5·3–5·6
Legumin pH	= 4·4–4·6
Globulin (yeast) pH	= 4·6
Globulin (carrot)	= 4·1–4·4
Globulin (tomato)	= 4·0

The iso-electric points of some leaf cytoplasmic proteins have been examined by Chibnall* and compared with the hydrogen-ion concentration of the leaf cell-contents:

	Iso-Electric Point of Protein	*pH of Cell-Contents*
Cabbage	4·7–4·0	5·6
Vicia Faba	5·1–4·3	5·69
Hogweed	5·0–4·3	6·19

It would appear from these figures that the proteins in the leaf cells of these plants are in solution on the alkaline side of their iso-electric points. In such circumstances, these proteins will be functioning as anions. If the hydrogen-ion concentration of the cell sap were to approach that of the iso-electric point of the proteins present, the latter would tend to be thrown out of solution with serious consequences to the life of the cell.

Some further investigations on the proteins of leaf cytoplasm have been made by Grover and Chibnall,† who claim that the protein present in the leaf cytoplasm is divisible into two fractions. One of these fractions represents protein which is in some kind of loose combination with substances soluble in alcohol, and the other fraction represents soluble proteins which are not in combination and pass into solution when the cytoplasmic gel is ground with water. This latter group of proteins belongs to the class of proteins known as globulins (see below). They show an iso-electric range from pH 4·0–5·0, and in all the cases that were investigated the leaf cell sap showed a higher pH than the range given above, so that these proteins are present in the cells of the leaf as anions.

THE CHEMICAL CONSTITUTION OF THE PROTEINS

On hydrolysis with strong mineral acids, the protein molecule is split up into a number of crystalline amino-acids. It is possible

* *J. Amer. Chem. Soc.*, 1926, **48**, 728. † *Ann. Bot.*, 1926, **40**, 491.

that some of the cleavage products are destroyed in the course of hydrolysis with strong acid, but usually approximately 80 per cent of the nitrogen present in protein can be recovered in the form of amino-acids.

The amino-acids are substances in which one or more of the hydrogen atoms (other than carboxyl hydrogen) are replaced by the amino-group —NH_2. They are amphoteric electrolytes and form salts with both acids and bases. They are resistant to the action of strong acids, even after prolonged boiling. All the amino-acids that have been isolated up to the present from natural sources possess the amino-group in the α-position, that is, the amino-grouping is attached to the carbon atom immediately adjacent to the carboxyl group. The general formula for the amino-acids isolated from protein hydrolysis may therefore be written: R—$CH(NH_2)$. COOH, where R represents the rest of the carbon framework. Amino-acids with two amino-groups in the molecule have also been obtained; these show basic properties, whereas amino-acids with two carboxyl groups in the molecule and only one amino-group show well-marked acidic properties. With the exception of the first member of the series, glycine or amino-acetic acid, CH_2NH_2 . COOH, all the amino-acids show optical activity.

The following list gives the best known amino-acids isolated from plant and animal proteins:

Glycine (α-amino-acetic acid)

$CH_2 . NH_2 . COOH$. M.P. 240° C.

d-Alanine (α-amino-propionic acid)

$CH_3 . CH . NH_2 . COOH$. M.P. 297° C.

l-Serine (α-amino-β-hydroxy-propionic acid)

$CH_2OH . CHNH_2 . COOH$. M.P. 293° C.

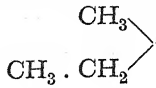
d-Valine (α-amino-isovaleric acid).

$$\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>\!CH . CHNH_2 . COOH. \quad \text{M.P. } 298^\circ \text{ C.}$$

l-Leucine (α-amino-isocaproic acid)

$$\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>\!CH . CH_2 . CHNH_2 . COOH. \quad \text{M.P. } 295^\circ \text{ C.}$$

d-Isoleucine (β-methyl β-ethyl- α-amino-propionic acid).



$CH_3 . CH_2 . CH(CH_3) . CHNH_2 . COOH$. M.P. 280° C.

l-Cystine (di[β-thio-α-amino-propionic] acid).

$COOH . CHNH_2 . CH_2 . S . S . CH_2 . CHNH_2 . COOH$

l-Aspartic acid (α-amino-succinic acid).

$COOH . CH_2 . CHNH_2 . COOH$. M.P. 270° C.

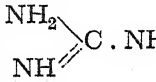
d-Glutamic acid (α-amino-glutaric acid).

$COOH . CH_2 . CH_2 . CHNH_2 . COOH$. M.P. 206° C.

d-Lysine (α-ϵ-diamino-caproic acid).

$CH_2NH_2 . CH_2 . CH_2 . CH_2 . CHNH_2 . COOH$. M.P. 252° C.

d-Arginine (δ-guanidine-α-amino-valeric acid).



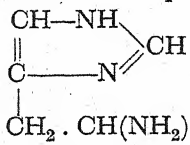
$NH_2 . C(=NH) . NH . CH_2 . CH_2 . CH_2 . CHNH_2 . COOH$

M.P. 207° C.

d-Ornithine (α-δ-diamino-valeric acid).

$CH_2NH_2 . CH_2 . CH_2 . CH_2 . CHNH_2 . COOH$

l-Histidine (β-imidazol-α-amino-propionic acid). M.P. 253° C.



$CH_2 . CH(NH_2) . COOH$

l-Phenylalanine (β-phenyl-α-amino-propionic acid). M.P. 283° C.

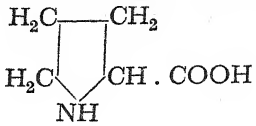
$CH_2 . CH(NH_2) . COOH$

l-Tyrosine (β-*p*-hydroxyphenyl-α-amino-propionic acid).

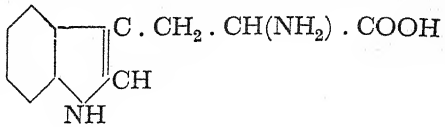
M.P. 314–318° C.

$CH_2 . CH(NH_2) . COOH$

l-Proline (α-pyrrolidine carboxylic acid).



l-Tryptophane (β-indole-α-amino-propionic acid). M.P. 289° C.



The amino-acids when treated with nitrous acid give a quantitative yield of free nitrogen, yet the proteins of which they are the direct hydrolytic products give but little nitrogen when treated with nitrous acid. It was therefore considered by Emil Fischer that the amino-acids in the protein molecule are linked together so that the amino-group of one acid is combined with the carboxyl group of another:

$$R—NH—CO—R_1$$

He was able to synthesize such compounds and termed them *peptides*. The simplest of these peptides is glycylglycine, formed by the union of two molecules of glycine,

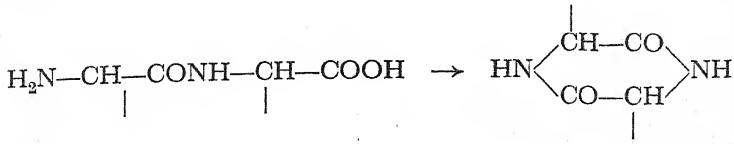
$$CH_2NH_2 . CO . NH . CH_2 . COOH$$

Peptides formed by the union of two amino-acids are termed dipeptides; when three molecules unite, the resulting product is termed a tripeptide, and the series has been extended so that many amino-acids are present, these products being termed polypeptides. The polypeptide with the highest number of amino-acids in the molecule synthesized by Fischer was eighteen, while Aberdalden has synthesized a polypeptide with nineteen amino-acids in the molecule.

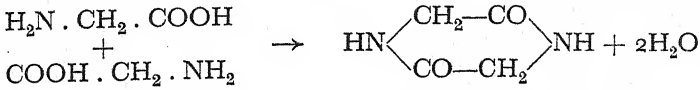
Peptides with a low molecular weight, i.e. di- and tripeptides, have in general the same properties as the amino-acids from which they are formed. They are crystalline, soluble in water and form insoluble copper salts. Peptides of high molecular weight are amorphous, form colloidal solutions in water and are precipitated by phosphotungstic and tannic acid like the proteins. In this respect they show an approach to the natural proteins.

The possibility that amino-acids are linked in other ways than

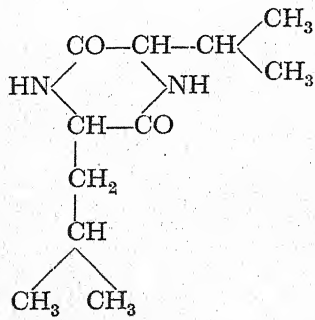
that described above, was envisaged by Fischer, who suggested that a ring structure of the following kind could be present:



The cleavage products of proteins, either by chemical means or by proteolytic enzymes, have frequently given products with a cyclic structure. For example, Sadikow and Zelinski* found that proteins heated with a 1 per cent solution of either sulphuric, hydrochloric or phosphoric acid in an autoclave at 180° C. gave rise to cyclic anhydrides of the amino-acids, the so-called diketopiperazines. The simplest diketopiperazine is glycine anhydride formed by the union of two molecules of glycine when an aqueous solution of glycine is allowed to stand for some time:

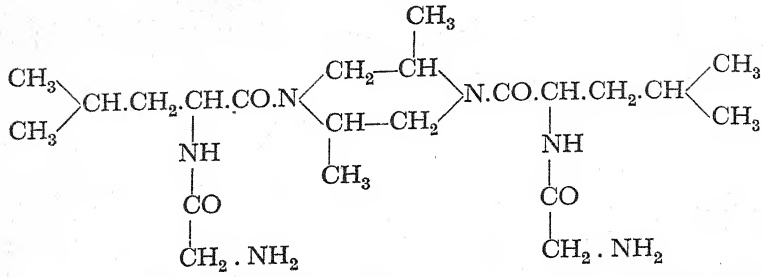


Abderhalden regards the protein molecule as a complex polymeride of these cyclic compounds, and he has been able to synthesize a number of new diketopiperazine derivatives. One such complex diketopiperazine derivative, isolated by Abderhalden from casein by hydrolysis with 5 to 10 per cent sulphuric acid, is *d*-valyl-leucine anhydride:



Abderhalden has also been able to synthesize these substances and has obtained such a complex compound as diglycyl-leucyl-2 : 5-dimethyl-piperazine:

* *Biochem. Zeit.*, 1923, **136**, 241; **137**, 397, 401.



Now none of the synthetic products prepared by Abderhalden were attacked by proteolytic enzymes, whereas certain of the synthetic products obtained by Fischer were hydrolysed by these enzymes. Osborne and Vickery* have pointed out this fact in a criticism of Abderhalden's view of the constitution of the proteins, and it is a large stumbling-block in the way of unqualified acceptance of this new idea of protein constitution in place of the old polypeptide hypothesis.

CLASSIFICATION OF PROTEINS

The scheme of classification given below is that of the American Committee on Protein Nomenclature. It is the most satisfactory scheme that is at present available.

I. *Simple Proteins.*
- (*a*) Albumins
- (*b*) Globulins
- (*c*) Glutelins
- (*d*) Prolamines
- (*e*) Albuminoids
- (*f*) Histones
- (*g*) Protamines

II. *Conjugated Proteins.*
- (*a*) Nucleoproteins
- (*b*) Glycoproteins
- (*c*) Phosphoproteins
- (*d*) Haemoglobins
- (*e*) Lecithoproteins

III. *Derived Proteins.*
- (i) *Primary protein derivatives.*
  - (*a*) Proteins
  - (*b*) Metaproteins
  - (*c*) Coagulated proteins

* *Physiol. Rev.*, 1928, 8, 393.

(ii) *Secondary protein derivatives.*
(*a*) Proteoses
(*b*) Peptones
(*c*) Peptides.

Among these various proteins, the glutelins and prolamines have only been isolated from plants and have not been found in animal tissues.

PROPERTIES OF THE PROTEINS

Albumins.—The proteins belonging to this group are readily soluble in water and coagulated by heat. They are not of frequent occurrence in plant tissues. Albumins are not precipitated from solution by the addition of sodium chloride or magnesium sulphate, but precipitation can be brought about by the addition of a saturated solution of ammonium sulphate. The following are the best known plant albumins: leucosin, which is found in the grain of wheat, rye and barley, legumelin, which occurs in cow-pea, soy bean and lentil, phaselin in the kidney bean and ricin in the seeds of castor bean (*Ricinus communis*).

Globulins.—The globulins are soluble in saline solutions, but insoluble in water. A number of the vegetable globulins have been obtained in the crystalline condition. They are precipitated from solution in 5 to 10 per cent sodium chloride by the addition of half-saturated ammonium sulphate. Plant globulins are only imperfectly coagulated by heating their saline solutions, and a few must be boiled for a long period before showing any change. Animal globulins, on the other hand, are readily coagulated. A long list of plant globulins have been isolated from seeds. A few are: legumin which occurs in the pea (*Pisum sativum*), broad bean (*Vicia Faba*) and lentil, edestin, which has been isolated from hemp seed (*Cannabis sativa*), maysin, found in maize (*Zea Mays*), vicilin found in pea, lentil, and broad bean.

Glutelins.—The proteins of this group are insoluble in water, saline solutions and alcohol. They are soluble, however, in weak alkali and acid. Glutelin forms part of the gluten of wheat.

Prolamines.—Proteins of the prolamine group are soluble in 70 to 90 per cent alcohol. They form one of the best characterized groups of plant proteins. On hydrolysis they yield large amounts of glutamic acid, proline and ammonia and small amounts of arginine and histidine, but practically no lysine.

Gliadin has been isolated from wheat gluten, hordein from barley grain and zein from maize.

Of the other groups of the simple proteins, no albuminoids or protamines have been isolated from plant tissues, and the evidence for the presence of histones is very slender.

Of the conjugated proteins, nucleoproteins, glycoproteins, haemoglobins, etc., there is at present no reliable evidence for the occurrence of these proteins in plant tissues. It is necessary, however, to discuss the nucleoproteins in detail, although their presence has been denied in plants.

As far back as 1868, Miescher was able to isolate from the nuclei of puss cells a substance which he termed "nuclein." This product was found to contain phosphorus and gave the usual protein colour tests. Later it was shown by Hoppe-Seyler and by Kossel that a similar product could be obtained from the red blood cells of birds and from the nuclei of yeast cells. A further step in the matter was made by Miescher himself, who isolated from the nuclei of the sperms of salmon a body which he considered to be a definite chemical individual, and stated that this was a salt of the protein protamine and an acidic body which he called nucleic acid. This was a considerable advance, and a large volume of research has since been directed on these products isolated from plant and animal nuclei, for it is possible that an understanding of their nature may lead to an understanding of the chemical structure of the nucleus.

Two special problems have arisen with regard to the constitution of this group of proteins. The first problem is the nature of the association of nucleic acid with protein, and the second is the constitution of nucleic acid itself.

The question of the association of protein and nucleic acid has led to a good deal of confusion in the past. It was considered that in the nucleus of the cell, a protein with basic properties combined with nucleic acid to give rise to the somewhat vague product "nuclein." Confusion was made worse confounded by the further supposition that nuclein itself was combined with proteins of the histone group to give some kind of complex. As far as animal tissues are concerned the position is now somewhat clearer, and the nucleoproteins may be regarded as being composed of various salts of basic proteins with nucleic acid, the proteins always being in excess.

Now nucleic acids have been isolated from plant cells, and the question has therefore arisen, Is there association of these acids with proteins of the same nature as in animal nucleoproteins? The problem is really one of words, as Jones* has stated: "The terms nucleoproteins, nuclein, and nucleic acid express a relation which means little more than that conveyed by the terms basic lead acetate, lead acetate and acetic acid . . . in reality 'nucleoprotein' means rather a 'method of preparation' than a chemical substance." It has now been shown by Hammarsten† that the nucleic acid isolated from thymus gland can combine with proteins in varying proportions.

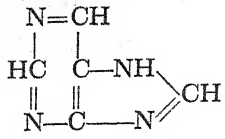
Nucleic Acids.—It will be seen from the above discussion that the chemistry of the nucleoproteins is really concerned with the nature of their specific acidic component, nucleic acid. There is no dispute with regard to the presence of nucleic acids in plant cells for they have been isolated from a number of different plant sources, notably yeast; the only question has been are they combined with proteins as nucleoproteins.

Nucleic acids isolated from either animal or vegetable sources, give, on ultimate hydrolysis, phosphoric acid, a carbohydrate or carbohydrate derivative and bases belonging to the purine and pyramidine group. Plant and animal nucleic acids, however, differ with regard to the nature of the sugar residues and the apparent variety of their bases. A comparison is given below of plant and animal nucleic acids:

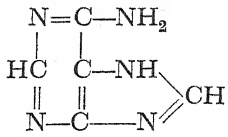
Plant Nucleic Acid	*Animal Nucleic Acid*
(1) Phosphoric acid.	(1) Phosphoric acid.
Pentose sugar (*d*-ribose).	(2) Laevulinic acid derived from a desoxyaldo-pentose (*d*-2-ribodesose).
(3) Purine bases.	(3) Purine bases.
Guanine.	Guanine.
Adenine.	Adenine.
(4) Pyramidine bases.	(4) Pyramidine bases.
Cytosine.	Cytosine.
Uracil.	Thymine.

The purine bases adenine and guanine are related to purine, which does not occur in nature.

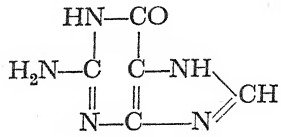
* *Nucleic Acids*, 2nd Edit., 1920.
† *Biochem. Zeit.*, 1924, **144**, 383.



Purine

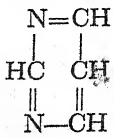


Adenine

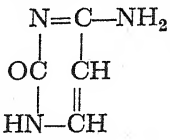


Guanine

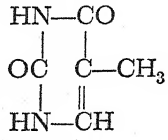
The constitutions and relationships to pyramidine of the three pyramidine bases cytosine, thymine and uracil, are shown below:



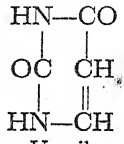
Pyramidine



Cytosine

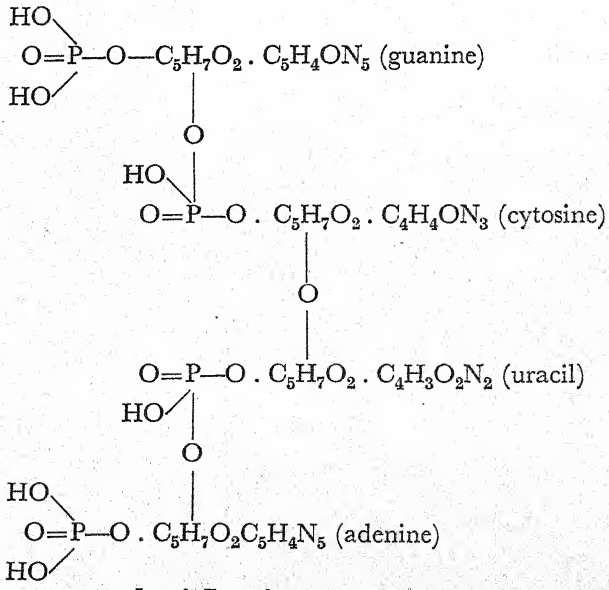


Thymine

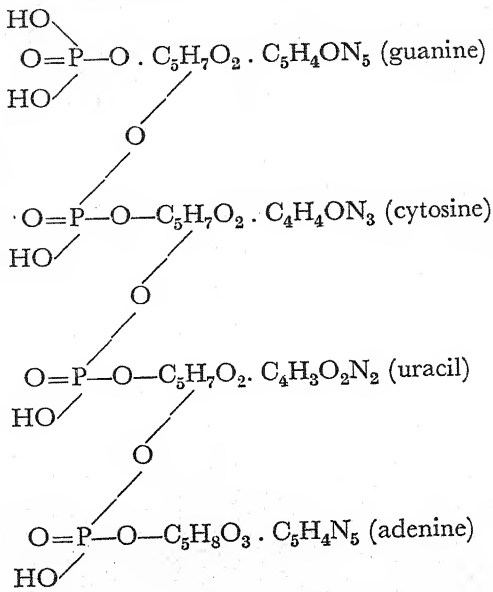


Uracil

With regard to the constitution of yeast nucleic acid, the following constitutions have been proposed by W. Jones and by Levene respectively. As far as can be decided at the present time there is more evidence for the structure proposed by Levene.



Jones's Formula



Levene's Formula.

Derived Proteins.—This is a term given to the products obtained by the hydrolysis of proteins. Of these hydrolytic products, the proteoses are soluble in water. They are not coagulated by heat, but the addition of saturated ammonium sulphate precipitates them from solution. The peptones, which are the products of still further hydrolysis of proteins, are also soluble in water, not coagulated by heat, and are not precipitated by the addition of a saturated solution of ammonium sulphate but give the biuret reaction. The peptides are the simplest products of protein hydrolysis and include polypeptides and individual amino-acids.

THE SOURCES OF NITROGEN SUPPLY FOR THE PLANT

The normal green plant, with few exceptions, obtains its supply of nitrogen for metabolic processes from the nitrogen present in the soil as inorganic salts. The Leguminosae are exceptional in this respect, and are able to make use of the free nitrogen of the atmosphere. Certain bacteria are also able to "fix" atmospheric nitrogen. The steps whereby molecular nitrogen is converted into complex organic nitrogenous products is not understood at present. The matter will be dealt with later in this chapter.

Nitrates and ammonium salts are the chief sources of nitrogen for the higher green plants. The question as to which is more efficient in this respect is still a matter of controversy and numerous publications have appeared and are still appearing on this problem.

The question is complicated by the difficulty of growing the plants under sterile conditions. All normal soils contain nitrifying organisms which are able to convert ammonium compounds into nitrates, a fact which vitiates a good deal of the older work on the subject.

It is a well-known fact that in high concentration ammonia is toxic to plants, and the pH of the medium appears to be of great importance in connection with the absorption of ammonium salts by plants. With increase of pH of the medium, there is an increased entry of ammonia, and injury may result from too great accumulation of this substance. The concentration of the ammonium salts is also of importance. It has been found by Mevius and Engel* that when the pH of the medium is between 5·3 and 5·6, high concentrations of ammonium salts can be used without injury to the plant. Two further factors must also be taken into account, namely, temperature and the supply of carbohydrates. Temperature plays an important part in the hydrolytic dissociation of ammonium salts, and the physiological effect of ammonium compounds is dependent to a high degree upon this hydrolytic dissociation and the corresponding tension of ammonia in the solution. The supply of carbohydrate is important because a supply is required for the synthesis of the amide asparagine (see below), which is apparently used by the plant to store toxic ammonia in a non-toxic form.

The crucial factor in this problem of nitrates versus ammonium salts appears to be the pH of the medium. If the pH of the medium be sufficiently low, the absorption of ammonium salts can be so retarded as to bring about actual nitrogen starvation. Provided the medium be sufficiently aerated, and the supply of carbohydrates adequate, and the pH of the medium does not fall below 6, ammonium salts appear to be as efficient as nitrates for the supply of nitrogen for the green plant.

It seems to be a fairly well-established fact that when the supply of nitrogen to a plant is in the form of ammonium salts, there is a rapid synthesis of amide nitrogen, amino-acids and other soluble

* *Planta*, 1930, 9, 1.

forms of organic nitrogen. On the other hand, when the plant is supplied with nitrogen in the form of nitrates, the synthesis of these substances is relatively slower and the amide content is not so high.

The age of the plant is also an important factor in this relation. According to Clark and Shive,* young tomato plants will absorb ammonium salts rapidly from a medium which is neutral or on the alkaline side, whereas nitrates are more readily absorbed when the medium is on the acid side; but in older plants, the pH of the medium does not influence the intake of nitrate to the same degree. For example, older material grown in a solution with a range of pH from 4 to 7, absorbed nitrate to a greater extent than ammonium salts at any pH. It was further found that, with the continued growth of the plant, absorption of nitrate nitrogen increased, whereas absorption of ammonium nitrogen fell.

SYNTHESIS OF PROTEIN IN THE PLANT

Since the proteins are the most important nitrogenous components of plants, it is natural that considerable attention has been paid to the possible ways in which these compounds may be built up. It must be mentioned here that the problem of synthesis of proteins by the plant is in a very speculative state.

There are two sides to the problem. There is first the question of *primary* protein synthesis, in which the plant is able to synthesize proteins from such simple initial products as nitrates or ammonium salts; and secondly, there is the question of *secondary* protein synthesis, in which the protein of reserve organs is first broken down to simpler products such as peptides and amino-acids, translocated in this form, and resynthesized to proteins in other parts of the plant.

Primary Protein Synthesis.—There are at present two views with regard to primary protein synthesis in the plant. According to one view there is in the first place individual synthesis of each amino-acid, and in the second place condensation of these various amino-acids to peptide peptones, proteoses and proteins.

Under laboratory conditions amino-acids have been synthesized by the action of ammonia upon aliphatic and aromatic acids. For example, when ammonia acts upon sorbic acid, which

* *Soil Sci.*, 1934, **37**, 203, 459.

is an unsaturated acid found in the unripe berries of the mountain-ash, diaminocaproic acid is formed:

$$\underset{\text{Sorbic Acid}}{CH_3 . CH = CH - CH = CH . COOH} + 2NH_3$$

$$= \underset{\text{Diaminocaproic Acid}}{CH_3 . CH_2 . CHNH_2 . CH_2 . CHNH_2 . COOH}$$

Following upon the initial stage of synthesis of amino-acids, there would have to be on this scheme, condensation of amino-acids to give rise ultimately to proteins. This second stage may be brought about under the agency of proteolytic enzymes, which may accelerate the synthetic side of the equilibrium.

The second view of protein synthesis in the plant is that simpler products than the amino-acids condense *en bloc* to give protein. The amide, asparagine, $COOH . CHNH_2 . CH_2 . CONH_2$, is considered to break down in respiration into ammonia and two-carbon residues. In the presence of such substances as pyruvic acid and acetaldehyde, there is the possibility that condensation *en bloc* may occur to give large polypeptide complexes.

The question of where primary protein synthesis takes place must be considered here. The evidence for the leaf being one centre is very strong, but there is also evidence that primary protein synthesis may take place in other parts of the plant. In the apple tree, for example, W. Thomas* has shown that nitrates are reduced in the finer roots. Similarly Nightingale and Schermerhorn† claim that reduction of nitrate occurs in the fibrous roots of asparagus and that organic nitrogen is elaborated in this region. Since a considerable amount of work has been carried out on the nitrogen relations of the leaf, it will be convenient to discuss some of the more important studies below.

Protein Synthesis in the Leaf.—The problem of protein synthesis in the leaf is complicated by protein hydrolysis and translocation. A diurnal variation in the total nitrogen-content of leaves has been described by several investigators. It was shown by Chibnall‡ that protein increases by day and falls by night in the leaves of *Phaseolus multiflorus*, while Maskell and Mason§ established a diurnal variation in total nitrogen in the leaves of the cotton plant,

* *Plant Physiol.*, 1927, **2**, 55, 67, 109, 245.
† *New Jersey Agric. Exp. Stat. Bull.*, 1924, **476**.
‡ *Biochem. J.*, 1924, **18**, 387, 395.
§ *Ann. Bot.*, 1929, **43**, 205.

and Barton-Wright and McBain* found a similar condition in the potato leaf. Presumably there must be synthesis by day, and hydrolysis and translocation by night, in the lamina of the leaf.

The fact that there is a diurnal variation in the nitrogen of the leaf blade raises the question as to whether light is a necessary factor for protein synthesis in the plant. It has been found that nitrates accumulate in leaves in the dark and disappear in the light, and in plants with variegated leaves it has been discovered that nitrates only disappear from the green portions.

It does not necessarily follow from this that protein formation is a photosynthetic process; it may be that carbohydrates are necessary for protein synthesis in the plant. There does appear to be some intimate relationship between carbohydrates and protein formation. It was found by Sapoznikow that as carbohydrates increased in the leaf through photosynthesis, proteins also increased, and Zalesski showed that protein synthesis could take place in leaves in the dark provided that there was an adequate supply of carbohydrates. It follows from these investigations that light only plays an indirect part in protein synthesis in the leaf. In the light, the leaf is able to build up carbohydrates and these are used in the synthesis of protein.

Muenscher,† using Chlorella, has brought forward some further evidence to show that light is not a necessary factor for protein synthesis in the plant. The alga was grown in nutrient solutions, and nitrogen was supplied in the form of calcium nitrate or ammonium sulphate. Cultures were kept in diffuse light and also in total darkness for periods up to 235 days. Quantitative estimations were carried out on volume, dry-weight and total nitrogen, and evidence was obtained that protein synthesis takes place in the dark when this plant is supplied with inorganic nitrogen.

The presence of potassium and of calcium appears to be essential for protein synthesis. Burrell‡ claims that, in the absence of potassium, beet forms less protein, and that there is an accumulation of amino-acids.

The initial substance for protein synthesis in the plant is nitrate. Nitrate is carried into the leaf blade in the transpiration current and there enters into the complex chemical reactions which eventually give rise to protein. The first step in the reaction

* *Ann. App. Biol.*, 1933.
† *Bot. Gaz.*, 1923, 75, 249.
‡ Ibid., 1926, 82, 320.

must be reduction of the nitrate, i.e. there must be conversion of the $—NO_3$ grouping of the nitrate molecule into the $—NH_2$ group, since it is in the latter form that nitrogen is found in the protein molecule. It is probable that the first stage in the reaction is conversion of nitrate to nitrite. This is an endothermic reaction, and energy is absorbed in the process, so that it can only take place by the transformation of other forms of energy, such as the transformation of radiant into chemical energy; or the necessary energy for this reaction may be obtained by the oxidation of previously formed reduced chemical compounds such as carbohydrates.

There is evidence for the presence of some kind of mechanism for the reduction of nitrates to nitrites in plant cells. In 1890 Laurent stated that he had found that the leaves of Sagittaria reduce nitrates to nitrites. This observation has been confirmed by Irving and Hankinson,* who claimed that there is enzyme present in the leaves of Iris, *Vicia Faba*, Elodea, and Potamogeton that is able to reduce nitrate to nitrite. Similarly Anderson† has shown that there is some kind of nitrate-reducing mechanism in a large number of plants. She found that when a few drops of a 4 per cent solution of sodium nitrate were added to the expressed sap of a plant, such as *Mercurialis perennis*, followed by a couple of drops of a 10 per cent solution of acetaldehyde, and the whole incubated at 45° C. for ten or twenty minutes, a positive test for nitrite is obtained. Eckerson‡ has shown that the expressed sap from various parts of the tomato will reduce nitrate to nitrite and finally ammonia. Whatever may be the nature of this reduction mechanism, as far as the tomato is concerned it appears to be thermostable, for Eckerson found that the reducing power of the sap is not destroyed when it is boiled, and reduction will still take place with boiled sap provided that the sap is maintained at a pH of 7·6. Lastly, the sap is able to bring about reduction of nitrate in the light or in the dark.

A number of schemes have been put forward to explain the steps in the conversion of nitrate to amino-acids. It has been suggested that the compound formhydroxamic acid,

$$\begin{array}{c} H—C—OH \\ \| \\ N—OH \end{array}$$

* *Biochem. J.*, 1908, **3**, 87.
† *Ann. Bot.*, 1924, **38**, 699.
‡ *Bot. Gaz.*, 1924, **77**, 377.

may be formed from nitrite and formaldehyde, and this by various intra-molecular changes gives rise to amino-acids. Actually nothing is known about the intermediate steps concerned in the process.

According to McKie,* who has followed Chibnall in this respect, asparagine is the chief source for protein synthesis in the green plant. It was found in lupin plants, grown for a period of 81 days, that the amount of asparagine increased rapidly to a maximum and then fell away, and as the asparagine decreased in amount a corresponding increase was found in the protein content. Whether asparagine does play any part in protein synthesis is a moot point, and further views on this subject will be discussed in subsequent sections.

SECONDARY PROTEIN SYNTHESIS AND PROTEIN DEGRADATION

Plants differ from animals in that there is no loss of nitrogen in the various processes of degradation and regeneration of protein that take place during active nitrogen metabolism. It will be necessary to discuss the problem of secondary protein synthesis and degradation from various different aspects. In the dormant seed, for example, there is reserve protein present, and on germination this protein must first of all be hydrolysed and then translocated to the newly developing organs. We have already seen that there is a considerable amount of evidence for considering the leaf to be one of the chief centres for primary protein synthesis. Protein synthesized in the leaf must be hydrolysed to soluble nitrogen products for translocation to the reserve organs, and once arrived there resynthesized to protein once more.

Nitrogen Transformations of the Germinating Seed.—In seeds the reserve proteins principally belong to the class of globulins, while in some of the grasses, the alcohol-soluble prolamines are present as well as glutelins.

At the onset of germination, the reserve proteins of the seed are hydrolysed to amino-acids prior to translocation to the newly forming organs. The cleavage of protein in germinating seeds takes place with rapidity. According to Jodidi,† 48 per cent of the protein present in the grain of *Zea Mays* is hydrolysed to water-soluble nitrogen compounds in eight days, while Zlataroff‡

* *Biochem. J.*, 1931, **25**, 2181.
† *J. Agric. Res.* 1925, **31**, 1149.
‡ *Biochem. Zeit.*, 1916, **75**, 200.

found that there is an increase in the amount of amino-acids and amides when seeds of *Cicer arietinum* germinate.

It has been shown by Schulze in the course of a large number of investigations that when the seeds of the Leguminosae germinate, there is a marked accumulation of asparagine. There is a greater accumulation of asparagine in seedlings grown in the dark than in the light, although considerable amounts of this amide are present in normal seedlings.

The high accumulation of asparagine found in leguminous seedlings by Schulze cannot be due to the presence of asparatic acid in the reserve protein of the seeds. For example, in the seedlings of *Lupinus albus* it was found that after eighteen days of growth, 25·7 per cent of the dry-weight was composed of asparagine. This is far more than can arise from protein hydrolysis alone.

In other plants, the amide glutamine,

$$NH_2 . CO . CH_2 . CH_2 . CHNH_2 . COOH,$$

was found in place of asparagine. This amide occurs notably in members of the Caryophyllaceae, many Cruciferae, and in the seedlings of Cucurbita and Ricinus. In Ricinus, Schulze found glutamine to accumulate on germination, but the amount was very considerably smaller than asparagine in leguminous seedlings, being only 2·5 per cent of the dry-weight.

Pfeffer, from certain micro-chemical tests, had found an accumulation of asparagine in seedlings grown in the dark, whereas, in the light, as carbohydrates accumulated from photosynthesis, asparagine disappeared. He advanced the view, which was considerably extended by Borodin, that since plants do not actively excrete their nitrogen like animals, and are extremely economical with their nitrogen in this respect, the function of asparagine in the plant body is that of a reserve substance into which protein nitrogen is converted for purposes of translocation. As long as carbohydrates are being synthesized during photosynthesis, asparagine is used for protein synthesis in the growing parts of plants, but when carbohydrate supplies are lacking, asparagine is held as a reserve.

It was found by Schulze that there is an increase of amide nitrogen at the expense of amino-acid nitrogen when hydrolysis of protein has ceased. The figures obtained by him for *Lupinus luteus* make this clear:

Per 100 Grams Ungerminated Seeds Without Testas

(*Percentage*)

	Protein N	*Asparagine N*	*Diamino Acid N*	*Other N*
6-day Seedlings ..	5·49	1·16	0·97	1·72
15-day Seedlings ..	1·71	4·02	1·22	2·39
24-day Seedlings ..	1·78	5·09	1·03	1·40

It has been shown by Palladin that oxygen is an important factor in protein decomposition. Although the process can take place in the absence of oxygen, it proceeds along different lines. In wheat seedlings it was found that on germination in the presence of oxygen asparagine accumulated, and little tyrosine or leucine was formed, but in the absence of oxygen there was considerable accumulation of tyrosine and leucine and practically no asparagine was formed.

Schulze collected a large mass of analytical data on the question of protein hydrolysis on seeds and seedlings and suggested that the nitrogen metabolism of the germinating seed takes place along the following lines: In the first place reserve protein undergoes hydrolysis, and a mixture of nitrogenous substances is produced. Asparagine, and in certain cases glutamine, are formed from this mixture of nitrogenous substances. The non-nitrogenous reserves of the seed, e.g. polysaccharides and lipides, are also hydrolysed and give rise to glucose and possibly other sugars. The next stage in the process is that glucose and amides are converted into proteins in the actively developing meristematic regions.

Prianischnikow has also conducted a long series of investigations into the nitrogen metabolism of the germinating seed. He was able to confirm Schulze's statement that asparagine accumulates on germination and that it owes its origin to amino-acids. He differs from Schulze with regard to the function of asparagine in the plant, and does not consider it to be a suitable substance for protein synthesis. Prianischnikow has taken up much the same attitude in this matter as Boussingault, who in 1864 suggested that asparagine functions in the plant in the same capacity as urea in the animal, i.e. that it is a safe combination for free ammonia, for if the latter were to be allowed to accumulate it

would be toxic to the plant. Animals continually excrete urea and require fresh supplies of nitrogen daily, whereas the plant stores its ammonia as asparagine, and this ammonia can be split off and used again for protein synthesis.

Nitrogen Transformations of Leaf and Shoot.—A diurnal variation in the nitrogen-content of the leaf is well established for a number of plants. Chibnall, who used the runner bean *Phaseolus multiflorus*, considered that there was a continuous decomposition of protein during the day as well as during the night, but the hydrolytic process was masked by the synthetic one in the light. He believes that asparagine, as the product of protein decomposition, serves as a translocatory substance for nitrogen. It is not at all probable that asparagine plays any part in the translocation of nitrogen. It has been shown by Maskell and Mason (see Chapter XII) that in the cotton plant there are steep negative gradients of asparagine nitrogen present in the stem, and this substance appears to act in some kind of storage capacity.

The important investigations of Ruhland and Wetzel* must now be discussed since they have an important bearing on the nitrogen relations of leaf and shoot. These investigators recognize two classes of plants, "amide" plants and "ammonia" plants. We have already had examples of "amide" plants and discussed the formation of asparagine. In the group of "acid" plants various organic acids, e.g. oxalic and malic are to be found. According to Ruhland and Wetzel, deamination of amino-acids gives rise to organic acids and ammonia; the precursors of malic and oxalic acid being the non-nitrogenous residues obtained from deamination of amino-acids.

Prianischnikow's views on asparagine formation have already been discussed, and it will be recalled that he considered that the function of asparagine was to bind toxic ammonia. In Ruhland and Wetzel's group of acid plants, there is present a high percentage of organic acids as well as ammonia in the form of ammonium salts. Ruhland and Wetzel were unable to find any correlation between the amount of organic acid and respiratory activity, and conclude that these acids are not products of respiratory activity. The acids and ammonia are both products of the same series of metabolic activities and the injurious effect of the ammonia is automatically compensated by the formation of acid, in other words, the ammonia is prevented from exerting

* *Planta*, 1926, **1**, 558; 1927, **3**, 765; 1929, **7**, 503.

any toxic action by being converted into the ammonium salt of the organic acid. The ammonium salts that accumulate in leaves during a period of darkness, disappear on the advent of light when carbohydrates are once more formed in photosynthesis, and it would appear that these nitrogenous compounds are then utilized in leaves for protein synthesis.

An extensive series of investigations have been carried out by Mothes* on the nitrogen fractions of various leaves. He showed that if the leaves were kept in the light, or fed with glucose in the dark, no amides were formed, and any amides that may have been present in the leaves originally disappeared under these conditions and were used in the synthesis of protein. If the leaves were placed for a prolonged period in the dark, so as to reduce the carbohydrate content, amides made their appearance and finally ammonia was also formed.

Mothes was able to confirm Palladin's statement that oxygen is necessary for amide formation, and if oxygen be a limiting factor, there is a marked increase in the concentration of amino-acids and basic nitrogen, but amides and ammonia are not formed. He was able to show that the oxidation of the products of protein hydrolysis in leaves takes place when the supply of carbohydrates is low, the end product of the oxidation process being ammonia. When, however, carbohydrates are present in suitable amount, asparagine is formed, and the ammonia is "stored" in this form.

The close connection between carbohydrate supply and asparagine formation was clearly brought out by these investigations. For example, whether the asparagine were produced in the plant or supplied from external sources, it always remained as such unless a suitable concentration of carbohydrates was available, when it entered into the synthesis of protein.

The behaviour of leaves with a high carbohydrate content when fed with ammonium salts was also examined. In such circumstances proteins were rapidly synthesized, but if the supply of carbohydrates were low, asparagine made its appearance, and when carbohydrates were absent ammonia was produced and poisoning of the leaves occurred. Finally, it was found that in the presence of various narcotics no amides were formed and frequently no ammonia.

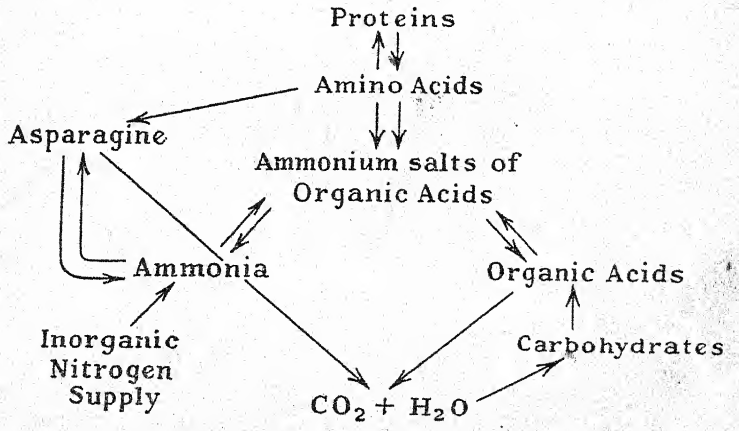
According to Kultzscher,† who has extended the observations

* *Planta*, 1926, **1**, 472. † Ibid., 1932, **17**, 699.

of Ruhland and Wetzel, plants with an acid sap, i.e. where the pH of the sap is less than 5, are able to store excess nitrogen in the form of ammonium salts of organic acids. This formation of ammonium salts is not merely a question of neutralizing toxic ammonia, but these salts are definite stores of excess nitrogen which can later be used for purposes of protein synthesis. Kultzscher is of the opinion that there is an equilibrium between amides and ammonia in the cell, and that in plants of relatively high acidity the equilibrium is shifted to the ammonia side, and ammonium salts are formed through the union of ammonia with organic acids. When amino-acids are deaminated, not only is ammonia released but acids are formed as well, and by the formation of ammonium salts, the ammonia and acids, as it were, take care of each other.

It is clear from the various investigations described above that plants are able to use a number of different methods for protecting themselves against the toxic action of ammonia. It is probable that the particular mechanism set into action will depend on a number of different factors, and as far as can be said at present, the most important of these factors appears to be the supply of carbohydrates, but other important factors are hydrogen-ion concentration of the cell sap, and the sources of the ammonia.

Engler has suggested the following outline as possibly representing the lines along which the various nitrogenous metabolites are formed in the plant:



The question of whether nitrogen is evacuated from leaves before leaf fall has been much debated. Thomas has made an

elaborate study of the nitrogen metabolism of the apple tree and found definite evidence that in the autumn, prior to leaf fall, the nitrogen of the leaf migrates into the stem and is stored in the first and second year shoots.

Synthesis of Protein in the Ripening Seed.—By far the largest amount of protein that occurs in plants is present in the seeds as reserve protein. A number of observations have been made from time to time with regard to how protein is formed in the ripening seed. It was shown by Zaleski* for maturing pea seeds that the amount of protein increased as amino-acids and nitrogenous organic bases decreased. This result is borne out by the following figures:

(*Percentage*)

Time	*Amino-Acids*	*Nitrogen Bases*	*Other Nitrogen Compounds*	*Protein*
Start	8·7	10·8	1·4	79·2
After 5 days ..	4·6	5·6	0·8	89·2

The question of protein formation in the wheat grain has been studied in detail by Woodman and Engeldow.† They were unable to find any trace of nitrate in the developing grain and showed that the nitrogen enters the grain as asparagine. Presumably the asparagine is oxidized to ammonia and forms the initial stage for protein synthesis.

NITROGEN ASSIMILATION BY THE LEGUMINOSAE

It has been known for many years that leguminous plants are able to grow normally and produce a good yield on soil which has not been treated with nitrogenous manures. The Romans were aware of the fact that leguminous crops in some way enriched the soil and made it more fertile. The work of Lawes and Gilbert at Rothamsted was conducted over a long period, and showed that when grain crops or leguminous crops are grown for a number of years on the same soil without addition of fertilizers, the nitrogen-content of the crop reaches a certain minimum value at which it remains constant. If, now, fertilizer containing

* *Ber. deut. bot. Ges.*, 1905, **23**, 126; *Beih. bot. Centralb*, 1911, **27**, 63.
† *J. Agric. Sci.*, 1924, **14**, 563.

no nitrogen was added, the nitrogen-content of the grain crop was not affected, whereas there was a well-marked increase in the nitrogen of the leguminous crop.

The classical investigations in 1888 of Hellriegel and Wilfarth on the nitrogenous nutrition of legumes showed that these plants are able to utilize the free nitrogen of the atmosphere. It had been shown many years before by Boussingault that legumes grown in well-sterilized soil (strongly ignited sand) were unable to assimilate atmospheric nitrogen, and failed to develop normally in the absence of nitrogenous fertilizers. Hellriegel and Wilfarth worked with both sterilized and unsterilized soils. In sterilized soils growth was checked on account of lack of nitrogen, whereas in unsterilized soils growth was normal. When a small amount of an infusion from unsterilized soil was added to sterilized soil, the growth of the plants was normal and they were found to be rich in nitrogen. Hellriegel and Wilfarth also brought to light two other important facts, firstly, that if the infusion from unsterilized soil were boiled before it was added to sterilized soil, the cultures remained stunted and showed no increase in their nitrogen, and secondly, that the infusion must be made from soil on which a leguminous crop had previously been grown. They also observed the presence of tubercles on the roots of leguminous plants that had been grown on unsterilized soils and found these to be absent when the plants were grown in sterilized soil.

It was concluded by Hellriegel and Wilfarth that legumes are able to assimilate free atmospheric nitrogen and that the tubercles on the roots of these plants are in some way directly concerned in the process, possibly through a symbiotic relationship between micro-organisms in the tubercles and the higher plant.

In cross-section, one of these tubercles shows a mass of parenchymatous tissue. On the outside, the tubercle is covered with a layer of cork, and the inner cells are thin-walled and contain bacteria, and have a high protein-content, while the outer cells contain little reserve material.

In the same year that Hellriegel and Wilfarth's investigations were published (1888), Beijerinck succeeded in isolating the bacteria from the tubercles and growing them in pure culture, and this work was further confirmed in 1890 by Prazmovsky.

Prazmovsky has called the bacteria in the tubercles of legumes *Bacillus radicicola*. It has now been shown that there are several

strains of this form. For example, *Robinia Pseud-acacia* when grown on soil without available nitrogen will only show normal growth when the soil is inoculated with cultures made from tubercles obtained from Robinia, whereas if an inoculation be made with cultures from pea or lupin tubercles normal growth fails to occur.

The bacteria effect an entrance into the plant through the tips of the root-hairs. Once inside the root-hairs they multiply rapidly and there is much softening of the walls of the hairs. They do not normally pass beyond the cortex of the root, and are kept

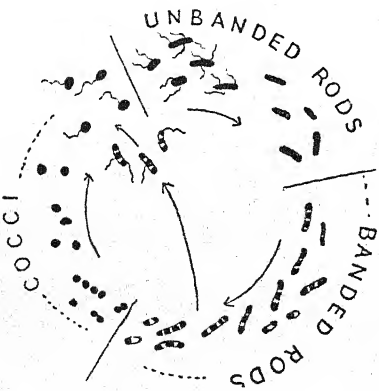


FIG. 26.—The life cycle of *Bacillus radicicola*. (After Thornton and Gangulee.)

confined within the inner cortical cells. The main vascular system of the root sends down strands into the tubercle and in this way connection is made with the circulating system of the plant.

It has been shown by Bewley and Hutchinson* that *Bacillus radicicola* passes through a very definite life-cycle. This life-cycle takes place in the host plant, in the soil and in artificial culture. The cycle commences with non-motile cocci which swell and develop flagellae, and invasion of the root-hairs by *B. radicicola* is brought about by these flagellated structures. The next stage in the cycle is the loss of the flagellae, and the bacteria once more become non-motile and vacuolated. This condition of the bacteria is found when they have arrived in the interior of the root-hair. Finally the bacteria show a banded appearance and then pass into cocci again or develop flagellae (Fig. 26).

* *J. Agric. Sci.*, 1920, **10**, 144.

It has been discovered by Thornton* that under certain conditions the bacteria can become parasitic in activity. If the host plant, for example, be grown in darkness, and the normal supply of carbohydrates to the nodules is cut off, the parasitic condition is developed, and the bacteria obtain their supply of energy requirements by consuming the host nucleus and cytoplasm and may even consume the cell wall.

The chemistry of nitrogen fixation is quite unknown and the various stages in the transformation of molecular nitrogen into complex organic protein still await solution.

Other plants besides legumes are known to possess the power of assimilating atmospheric nitrogen. In certain of the tropical Rubiaceae, numerous rounded thickenings occur on the leaves which contain bacteria. These bacteria (*Mycobacillus rubiacearum*) fix free nitrogen in the same way as *B. radicicola* found in the tubercles of leguminous roots.

NITROGEN ASSIMILATION BY BACTERIA

Certain soil bacteria possess the power of fixing molecular nitrogen. It was found in 1893 by Winogradsky that when he inoculated a solution of glucose with ordinary garden soil, vigorous fermentation took place with evolution of carbon dioxide, and considerable hydrogen, and the formation of acetic and butyric acids. At the same time fixation of atmospheric nitrogen took place. He showed that the amount of nitrogen fixed in the course of this process was related to the amount of sugar utilized. On an average for every 2 to 3 mgs. of nitrogen fixed by these bacteria 1 gm. of glucose was decomposed. The organism concerned here was called by Winogradsky *Clostridium pasteurianum* and is an anaerobic form living in the absence of free oxygen. It was considered by Winogradsky that the nascent hydrogen produced from the decomposition of glucose might by direct combination with nitrogen give ammonia. This suggestion was based on the fact that ammonium salts inhibit the fixation of nitrogen, but increase in the concentration of glucose counteracts this inhibitory effect. Increase of the glucose concentration would lead to the removal of the ammonia to supply nitrogen for fresh growth of the cells.

A second nitrogen-fixing bacterium, *Azotobacter chroococcum*, was

* *Proc. Roy. Soc.* (*Lond.*), 1929, **104B**, 481; 1930, **106B**, 110.

isolated in 1901 by Beijerinck. This is an aerobic form and is able to fix 10 mgs. of nitrogen for every gram of glucose oxidized. Carbon dioxide is the chief decomposition product formed by the action of *Azotobacter chroococcum* on glucose, but ethyl alcohol, as well as acetic, lactic and formic acids are also produced.

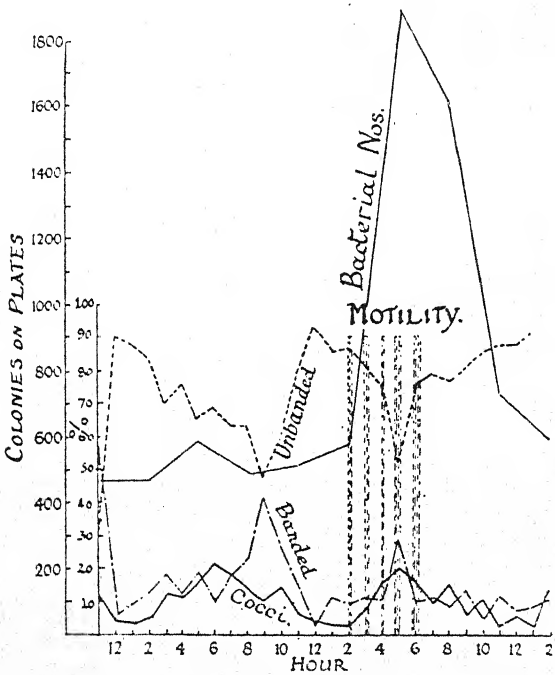


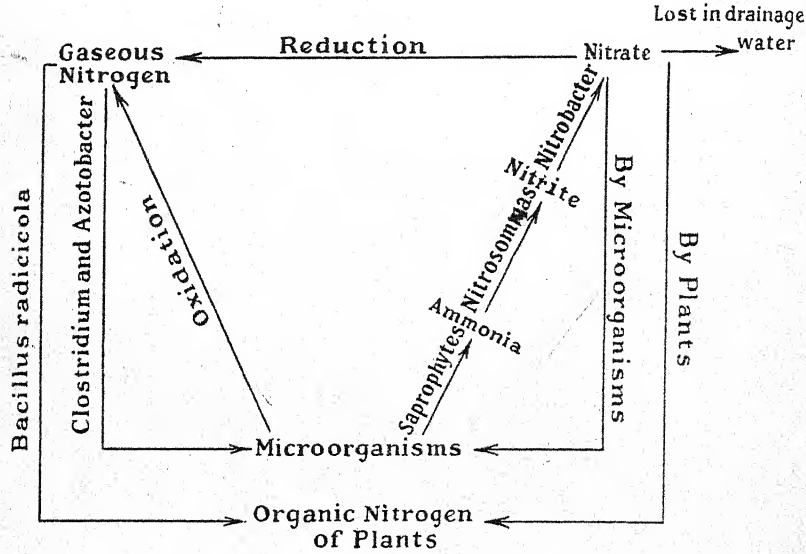
Fig. 27.—Curves showing the distribution of the total number of organisms, as well as the percentages of cocci, banded and unbanded rods, estimated at two-hourly intervals in culture. (After Thornton and Gangulee.)

NITRIFICATION OF THE SOIL

The formation of nitrates in the soil from the remains of dead animals and plants is dependent upon the activities of various micro-organisms. The complex organic nitrogenous compounds present in dead remains are first of all broken down to ammonia and ammonium salts through the agency of saprophytic micro-organisms. It was shown by Winogradsky that the ammonia and ammonium salts are first of all converted into nitrites by the action of a bacterium known as Nitrosomonas. In the American continent another genus, Nitrosococcus, carries out this function.

Nitrites formed under the agency of Nitrosomonas or Nitrosococcus are next oxidized to nitrates by the bacterium Nitrobacter. These are all aerobic forms and need oxygen. It was found by Winogradsky and Omeliansky that the addition of such organic compounds as glucose, peptone or asparagine checked the growth of these forms, and it has been found that like green plants they are autotrophic organisms and obtain their supplies of carbon from carbon dioxide. The energy set free in the oxidations that lead to nitrification is used for the chemosynthesis of carbohydrates from carbon dioxide and water.

Loss of nitrogen may occur in richly cultivated soils through the agency of *Bacillus denitrificans* which oxidizes nitrogenous substances to free nitrogen. The nitrogen cycle in nature is shown diagrammatically below.



CHAPTER XI

ASH

It has already been seen that plant tissues contain the elements carbon, hydrogen, oxygen and nitrogen. In the green plant, carbon, hydrogen and oxygen are obtained from the carbon dioxide of the air and water absorbed from the soil, and nitrogen is also supplied from the soil either in the form of nitrates or ammonium salts. Other elements are also found in plant tissues, for on incineration a residue of ash is always left. A large number of elements have been isolated from this ash, and Palladin enumerates a list of 31 elements that have been recognized in plant ash.

The pioneer investigations of Boussingault and Salm-Horstmar between the years 1840 to 1860 showed that the higher plants were able to grow on such media as sand, quartz, ground pumice or even sugar charcoal which had been well boiled with acid, thoroughly washed with distilled water, and then watered with mineral salt solutions of known composition. In 1860 Sachs introduced the method of water-culture, and grew a variety of land plants, such as *Zea Mays*, to maturity in solutions containing only dissolved salts and no organic matter.

The importance of these investigations lies in the fact that they showed that green plants could be made to complete their life-cycle without being supplied with organic matter from the environment. On the old humus theory, the plant was supposed to obtain its carbon supplies from the humus of the soil. These experiments with sand and water-cultures led to the establishment of the fact that the sole source of carbon for the green plant is the carbon dioxide of the atmosphere. Liebig in 1840 had advocated such a view, and put forward a mineral theory of nutrition for plants. His advocacy of his own views was very violent and he dealt with his opponents with much vituperation. Unfortunately for him, when he attempted to put his theories into practice his mineral mixture proved a complete failure. The general soundness of his views, however, was later proved by the investigations described above of Boussingault, Salm-Horstmar, Sachs and others.

From numerous investigations with sand- and water-culture

it has been shown that, besides carbon, hydrogen and oxygen, plants require the following elements for successful growth: nitrogen, phosphorus, sulphur, potassium, magnesium, calcium and iron. When any one of these elements is deficient in amount normal growth is prevented and the plants become stunted and die before flowering. These form the so-called "essential elements" for plant development. Investigations carried out over the last twenty years have shown that besides the "essential elements" minute amounts of certain other elements, such as boron, must be present for healthy growth of plants.

There are a large number of formulae available for the preparation of these culture solutions. Sachs used a mixture of the following composition: KNO_3, 1 gm.; $Ca_3(PO_4)_2$, 0·50 gm.; $MgSO_4$, 0·50 gm.; $CaSO_4$, 0·50 gm.; NaCl, 0·25 gm.; $FeSO_4$, trace; Water, 1,000 c.c. Knop's culture solution has the composition: $Ca(NO_3)_2$, 0·80 gm.; KNO_3, 0·20 gm; KH_2PO_4, 0·20 gm.; $MgSO_4$, 0·20 gm.; $Fe_3(PO_4)_2$, trace; water, 1,000 c.c.

A number of investigations were carried out in America using a three-salt solution. Shive,* for example, used Knop's solution, plus a trace of iron, but left out the potassium nitrate. Three different total concentrations of this mixture were employed, the osmotic pressures of which were 0·1, 1·75 and 4·0 atmospheres. The solution with an osmotic pressure of 1·75 gave optimum growth for wheat and buckwheat. For further details regarding the necessary proportions and concentrations of salts on plant growth see Tottingham.† The monograph by Russell, *Soil Conditions and Plant Growth*, should also be consulted.

For successful results with water-cultures, the following conditions must be observed. The solutions must be well aerated. Various devices have been used for the proper aeration of culture solutions, such as that of Allison.‡ The solutions must be continually renewed. It was found by Allison and Shive§ that soy beans grown in water-culture or in sand in which the solution was renewed continuously at the rate of 1·1 litres daily gave plants in every way superior to those grown in cultures which were only renewed at intervals. The culture vessels must be protected from light to prevent growth of algae. The best containers are those made of porcelain.

* *Amer. J. Bot.*, 1915, **2**, 157.
† *Physiol. Res.*, 1914, **1**, 133.
‡ *New Jersey Agric. Exp. Stat. Ann. Report*, 1921, 338.
§ *Amer. J. Bot.*, 1923, **10**, 554.

THE PHYSIOLOGICAL RÔLE OF SINGLE ELEMENTS IN PLANT NUTRITION

Although it is recognized that the various essential elements required for plant growth fulfil important and specific rôles in metabolism, it is not yet known with any degree of certainty how these elements act in living cells, nor is it known in all cases in which compounds each mineral element occurs in plants and what changes these substances undergo in living cells. Each element must play some specific rôle in metabolism, although some have several activities thrust upon them, e.g. potassium, which is somewhat of a Pooh Bah in this respect. The specific importance of mineral elements in plant nutrition is shown by the fact that they cannot be replaced by others. For example, attempts have been made to replace potassium by either sodium, rubidium or caesium; magnesium by beryllium; calcium by either barium or strontium, with no success. It is therefore evident that the plant requires these individual elements for its metabolic activities, but the exact way in which they are used in the living cell is still far from clear.

A few of the more important investigations on the part or parts played by different mineral elements in plant growth are described below.

Nitrogen.—The source of nitrogen supply to the plant has already been discussed in Chapter X, and the problem of nitrates *versus* ammonium salts briefly considered. It is well known that nitrogenous manures are very rapid in their action and in large amounts lead to rank and lush growth. When wheat is supplied with heavy dressing of nitrogenous manures it shows a susceptibility to infection from rust. It has been found that sclerenchymatous tissue is reduced and the collenchyma increased, and this condition is favourable for invasion by Puccinia, for the mycelium of this fungus is only able to grow in the collenchymatous tissues.

Gregory* has investigated the influence of nitrate on barley and found that, although there is an increase of leaf area with addition of nitrate, there is no increase of the assimilation rate, but the addition of potassium and phosphorus increases not only leaf area but also assimilation rate.

H. L. White† has examined the influence of nitrogen manuring on

* *Ann. Bot.*, 1926, **40**, 1.

† Ibid., 1936, **50**, 403.

the aquatic *Lemna minor*. The intensity of light and the temperature were kept constant. It was found that severe nitrogen starvation led to the production of a high dry-weight per unit area, short root, high starch-content and high net assimilation rate, but the frond area was small, the rate of respiration was low, so too was the multiplication rate, and the protein-content and chlorophyll-content was also low.

In moderately low concentrations of nitrogen, a long root was developed and a steady multiplication rate, but this fell later. Dry-weight per unit area and net assimilation rate were high, while the frond area was again low. When the concentration of nitrogen was increased over the optimal amount, neither the net assimilation rate, the multiplication rate, nor the dry-weight per unit area were affected, but the root was short as in the case of severe nitrogen starvation and the frond area was also low.

A characteristic of nitrogen starvation found in *Lemna minor* was the high carbohydrate-content. White suggests that under conditions of nitrogen starvation there is less amylolytic activity, leading to a reduced rate of respiration and an accumulation of carbohydrate, and that the falling multiplication rate will also tend to lead to an accumulation of carbohydrates owing to their reduced consumption in metabolism.

Sulphur.—As far as the higher green plants are concerned, sulphur can only be taken in as sulphate. Sulphites and thiosulphites cannot be utilized. It is a remarkable fact that only the highest oxide of sulphur can be used by the green plant, for in the cell sulphates are reduced completely. In the proteins sulphur is never found in union with oxygen. In the Cruciferae sulphur is found in various volatile forms, such as mustard oil, allyl sulphide and mercaptans. Some of these compounds have also been isolated from the Liliaceae (garlic and onion). These compounds occur in the plant in the form of glycosides.

Phosphorus.—Plants are only able to absorb phosphorus as phosphate, although it has been reported that the higher plants can utilize organic phosphorus such as nucleic acid. Phosphates, unlike the sulphates, do not undergo any great change in the plant. In the lipins it is still present as orthophosphate, and in alcoholic fermentation it occurs as hexose mono- and di-phosphates (see Chapter XIII).

The physiological function of phosphorus is difficult to discover. It appears to be necessary for nuclear division. This may

be because it forms an integral part of the nucleus. It is necessary for the formation of the lipins and nucleic acid which are present in nearly every living cell. The addition of phosphorus fertilizers promotes root growth and the earlier ripening of grain crops.

The importance of phosphorus to the plant lies in its early application. It has been shown by Brenchley* that full development of barley will take place, shown by the number of tillers, ears and grain produced, provided that the plants are supplied with phosphate over the first six weeks of growth. On the other hand, if the plants were deprived of phosphate in the early stages of growth, the ultimate amount of phosphate taken up showed a rapid fall.

It has been shown by F. J. Richards and Templeman† that in barley plants suffering from phosphorus deficiency, the amount of protein was rapidly reduced, even in the early stage of leaf development, and this decline continued with age. A further very characteristic feature of phosphorus starvation that was discovered was the high concentration of amide. It would appear from this investigation that lack of phosphorus checks protein synthesis in barley and as a result there is a marked accumulation of amide and it is possible that this fact may account for the many features of similarity shown by plants suffering from phosphorus deficiency and nitrogen deficiency. In any event, either phosphorus or nitrogen deficiency leads to a fall in meristematic activity.

Potassium.—An extraordinary range and variety of functions have been assigned to potassium in the plant. A few of these activities will be described below, but it is certainly very curious why potassium should play such an important rôle in plant metabolism, for it is known not to enter into any organic complexes and occurs in the ionic condition in the cells.

The outward signs of leaves receiving an insufficiency of potassium is very characteristic. They give the appearance of being scorched at the tips, and the edges become dull in colour.

A survey of the distribution of potassium in the potato plant has been carried out by Penston,‡ who used microchemical tests for this purpose. She discovered potassium to be localized in the cytoplasm and vacuoles, but found it to be absent from the nucleus. Potassium appears to have a wide distribution in the potato, and was found in all living cells, and was especially abundant in the apical meristems of shoot and root.

* *Ann. Bot.*, 1929, **43**, 89. † Ibid., 1936, **50**, 367. ‡ Ibid., 1931, **45**, 673.

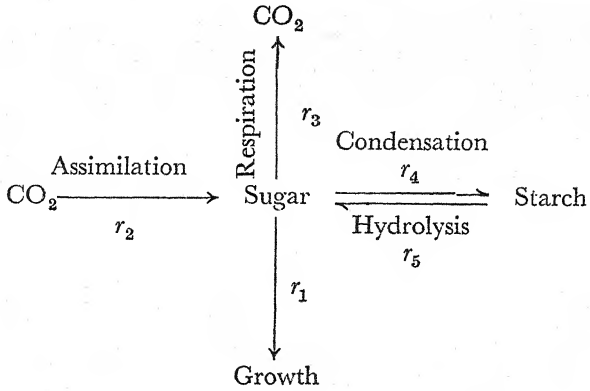
The presence of potassium appears to be necessary for the normal formation and translocation of carbohydrates. It has been found that in such plants as sugar beet, mangold and potato, which are large formers of carbohydrate, deficiency of potassium leads to a fall in the growth of leaves and stems. The older investigations indicated that potassium was necessary for starch formation, but this statement has been contradicted by Smith and Butler.* Maskell,† who has carried out a series of observations on the formation of starch in the potato leaf under field conditions, found that plots which had received potassium sulphate showed an increase of starch formation over plots which had received their potassium in the form of the chloride. Similarly, Janssen and Bartholomew,‡ who investigated a number of different plants in connection with carbohydrate formation and presence of potassium, found that there is a close relationship between the percentage of potassium and percentage of carbohydrate formed in such plants as soy bean, cow-pea, cotton and oat.

A careful examination of the function of potassium in the metabolism of *Lemna minor* has been made by H. L. White.§ The plants were grown under a constant light intensity and at constant temperature in mineral culture solution containing varying amounts of potassium. When no potassium was added to the culture solution used, it was found that the rate of increase of the frond numbers fell away with time. The colonies in the no-added potassium solution showed an abnormally high starch-content. When, however, as small an amount as 1 mg. of potassium was added to the solution there was only a moderate amount of starch in the frond cells. Other characteristics shown by the potassium-starved plants were, high dry-weight per unit area and a low net assimilation rate. The most remarkable feature of the recovery from potassium starvation shown by Lemna was an immediate increase in frond area, which was accompanied by a fall in starch-content.

This fall in the starch-content is presumably to be associated with a high concentration of soluble sugars. White has put forward various suggestions to explain this result. For example, the starch $\rightleftharpoons$ sugar equilibrium of the frond cells may be in-

* *Ann. Bot.*, 1921, **35**, 189.
† Ibid., 1927, **41**, 327.
‡ *J. Agric. Res.*, 1929, **38**, 447; 1930, **40**, 243.
§ *Ann. Bot.*, 1936, **50**, 175.

fluenced by potassium. In the next place, potassium may affect the real assimilation rate, the effect being restricted to colonies grown with no-added potassium in which the chlorophyll-content is reduced. These various suggestions are schematically shown below:



On this scheme the sugar concentration is considered to be regulated by the levels of five resistances in the way of possible gain or loss of sugar. By growth, sugar will be lost, it will also be lost by condensation to starch and by respiration. Sugar will be gained by photosynthesis and hydrolysis of starch. When the plants are supplied with the full amount of potassium an equilibrium between starch and sugar will be present.

Since the relative rate of multiplication of the fronds falls away under conditions of potassium starvation, less sugar will be used in growth (resistance r_1 in the above scheme). The net assimilation rate is also low and White suggests that the real assimilation rate will at the same time be reduced (r_2). The decreasing amount of synthesis of sugars by the fall in the real assimilation rate may offset the decreasing utilization of sugars in frond multiplication. If an increase in the resistance of r_2 be approximately counterbalanced by an increase in the resistance r_1, the net concentration of sugars should be maintained appreciably unchanged. The starch $\rightleftharpoons$ sugar equilibrium may be determined by the remaining resistances, r_3, r_4 and r_5 so that high starch-content and high dry-weight per unit area is quite compatible in potassium-starved fronds with a low net assimilation rate. It was found that respiration (r_3) does not enter into the problem to any major extent, because a high rate of respiration would lead to a loss of

sugar and presumably to a greater hydrolysis of starch to make up this loss.

Direct measurements of starch hydrolysis by potassium-starved plants were made and it was found that the rate was much retarded. When a potassium-starved colony was transferred to a full potassium solution there was an immediate fall in the starch-content. This fall in the starch-content was discovered to take place before there was a recovery in the net assimilation rate or multiplication rate of the fronds. It is evident that this result must be associated with an increased rate of hydrolysis of starch in the presence of adequate potassium.

It would thus appear that the major rôle of potassium in the metabolism of *Lemna minor* is to regulate the carbohydrate metabolism through control of the starch $\rightleftharpoons$ sugar equilibrium, by some kind of activation of the enzymes concerned in the hydrolysis and synthesis of starch.

According to Gregory and F. J. Richards* deficiency of potassium leads to a subnormal rate of photosynthesis in barley, whether the plants be grown under conditions of high or low light intensity, whereas the rate of respiration is supernormal. The reason for the rise of the respiration rate when potassium supply is deficient is not known. A variety of suggestions has been put forward to account for this fact, such as increase of amino-acids in potassium-deficient leaves leading to an increased rate of respiration. There is a certain amount of evidence available to show that increase of amino-acid content does lead to a rise in the rate of respiration.

Nightingale† and his co-workers found that deficiency of potassium leads to the symptoms typical of initial nitrogen starvation in tomato plants. It was also found that nitrates were abundant in all parts of the plants and that this high concentration of nitrates was maintained up to the time of the death of the plants. Nightingale has suggested that potassium is essential for the synthesis of organic nitrogen from nitrates. It was shown, for example, that the addition of potassium to the minus potassium tomato plants led to the production of considerable amounts of nitrites in the phloem, as well as the cortical tissues of stem and veins. Penston, working with potato, is also of the opinion that potassium is an important factor in the synthesis of protein by plants.

* *Ann. Bot.*, 1929, **43**, 119. † *New Jersey Agric. Exp. Stat. Bull.*, 1930, **49**, 9.

Another function of potassium in the plant that has been suggested is that of succulence. It was considered by Janssen and Bartholomew that plants grown in the presence of large amounts of potassium were more succulent than plants grown on a nutrient solution containing a small amount of potassium. Nightingale, however, has contradicted this statement, whereas Tincker and Darbishire* found that plants of *Stachys tuberifera* deprived of potassium wilt more easily than those grown in the presence of this element. On the other hand, Gregory and Richards,† and also Richards,‡ are quite emphatic on the point that potassium deficiency leads to an increase of succulence. This divergence in views on the question can scarcely be due to differences in the plant material employed. It is possible that the explanation may lie in the varying nature of the nutrient media used by these different workers.

Calcium.—This is an essential element for all chlorophyll-containing plants. It is not, however, required by the fungi. In the higher plants, lack of calcium leads to discolouration of the roots and brown spotting and later death of the leaves.

A number of different rôles in plant metabolism have been assigned to calcium from time to time. The older physiologists considered that it played some important part in the synthesis of proteins. More recent investigations have, on the whole, tended to confirm this view. Parker and Truog,§ for example, have suggested that the association between calcium and protein is due to the precipitation of acids formed as by-products in protein synthesis as calcium salts. Nightingale|| and others have found that deficiency of calcium in the tomato leads to deficiency of nitrate absorption by the roots. This fact may be one explanation of the close relationship that has generally been found between protein synthesis and calcium. The discovery by Chibnall that the nitrogen-free lipin, phosphatidic acid, occurs as the calcium salt in various leaves may have an important bearing on the problem of the function of calcium in plant nutrition. If, as he has suggested, this substance forms an integral part of the protoplasm (*élément constant*) of plants, calcium probably plays some important and not understood rôle in the vital activity of the leaf (see Chapter IX).

Magnesium.—This metal occurs in plants in much smaller

* *Ann. Bot.*, 1933, **47**, 27. † Ibid., 1929, **43**, 119. ‡ Ibid., 1932, **46**, 367.
§ *Soil Sci.*, 1920, **10**, 49. || *Plant Physiol.*, 1931, **6**, 605.

amounts than either potassium or calcium. It will be recalled that magnesium forms an integral part of the molecules of chlorophyll *a* and *b*. Magnesium is more abundant in seeds and leaves than in other parts of the plant. It is also more abundant in oily seeds than in starchy seeds. The fact that it is more abundant in oily seeds than starchy is probably due to its presence as the magnesium salt of phosphatidic acid. It was shown by Chibnall and others that in seeds this acid is present in the form of the magnesium salt and on germination the magnesium salt is converted into the calcium one and in leaves the acid is present as calcium phosphatidate (see Chapter IX).

SECONDARY ELEMENTS IN PLANT NUTRITION

A long series of investigations conducted over the last twenty years have shown that besides the elements enumerated above as being essential for normal plant growth, other elements in minute amounts are also necessary. It has been found that when plants are grown in the usual culture solutions prepared from highly purified chemicals, many fail to give normal growth. When, however, small amounts of certain elements such as boron, zinc, aluminium, and manganese are added, normal growth takes place.

Iron.—Green plants are unable to develop chlorophyll in the absence of iron, yet this element does not enter into the composition of the chlorophyll pigments. The amount needed by the green plant is very small. According to Gile* less than 1 part per 10,000,000 is needed by rice plants to show healthy normal growth. Plants are able to assimilate ferrous and ferric salts equally well. The function of iron in the economy of the green plant is not known. It is known, however, that plants in the absence of iron show chlorosis and that it is necessary for the formation of the chlorophyll pigments. It has been suggested that iron plays some catalytic rôle in the cell since it is required in such minute quantity. It is possible that it may have an important function in some of the oxidative processes of the cell (see Chapter XIII).

Boron.—The presence of boron for the healthy development of a large number of plants has now been completely established, and the list of plants requiring small amounts of this element has continually been extended.

Warington† and others have carried out an extended series of

* *J. Agric. Res.*, 1916, **7**, 503.

† *Ann. Bot.*, 1923, **37**, 629; 1926, **40**, 27.

investigations at the Rothamsted Experimental Station on the effect of boron on different plants. It was found that the broad bean (*Vicia Faba*) will only attain full development in the presence of a trace of boron, and no other element can replace it. In the absence of boron the bean plants become very stunted and die in a characteristic manner, the tips blackening and withering. The amount of boron required for normal development is very small, optimum results being obtained with concentrations of the order of one part in a million, while larger concentrations (1 in 5,000) were definitely toxic.

Boron is essential for the formation of the root nodules of *Vicia Faba*. According to Brenchley and Thornton,* in the absence of this element the nodules are much reduced in size and the bacterial population is also reduced in amount and change their normal symbiotic habit for active parasitism and attack the host protoplasm.

A number of attempts have been made with no very great success to ascertain the precise function of boron in plant metabolism. Brenchley and Warington† showed that in *Vicia Faba* the need of boron is independent of the pH of the medium in which the plants are growing, and that it apparently plays some important part in the calcium metabolism of the plants, for in the absence of boron *V. Faba* is unable to assimilate calcium. It is unlikely that boron plays a catalytic rôle in plant metabolism, for it has been established by Johnston and P. L. Fisher‡ that the tomato requires a continuous supply of boron throughout its life-cycle for healthy growth to be maintained.

Manganese.—This element has a very wide distribution in plants and is considered to be essential for the healthy growth of a number of plants. According to McHargue,§ manganese is as important as iron in the formation of the chlorophyll pigments. The highest concentration of manganese is said to occur in leaves.

Zinc.—It has been known for some years that zinc is a powerful stimulant of growth of such micro-organisms as Aspergillus. There is now a certain amount of evidence that the absence of zinc causes "deficiency diseases" in Citrus. It has been suggested that the disease commonly called "little leaf" and another known as "mottle leaf" may be due to zinc deficiency.

* *Proc. Roy. Soc.* (*Lond.*), 1925, **98B**, 373.
† *Ann. Bot.*, 1927, **41**, 167.
‡ *Plant Physiol.*, 1930, **5**, 387.
§ *J. Ind. Eng. Chem.*, 1926, **18**, 172.

CHAPTER XII

THE TRANSPORT OF SOLUTES

It has already been emphasized that the green plant is a synthetic machine and is able to elaborate its food material from such simple initial products as carbon dioxide, water and dissolved salts. In unicellular plants, for example Chlamydomonas, these elaborated food materials are used directly in the life and work of the cell, but the higher plants are specialized structures, and metabolic products formed in one part have to be transported to another for different purposes. Thus, in the higher plants, leaves are the chief centre of carbohydrate synthesis and possibly of protein synthesis as well, and these products must be translocated out of the leaves to the storage organs and also to fresh centres of development. Moreover, water and dissolved salts are obtained from the soil by the root system and transported through the plant to the leaves and other regions. It is therefore evident that channels must exist in the structure of the higher plants capable of carrying out these functions of translocation.

In unicellular plants, the question of translocation does not arise; in such organisms as Spirogyra and Ulothrix substances have to be moved for only small distances, specialized channels are therefore not necessary for transport, but in the higher plants materials have in many cases to be moved for long distances and there are specialized channels to facilitate this transport.

In 1671 it was found by Malpighi that if a ring of bark were removed from a woody stem, the portion above the wound grew vigorously and developed fresh roots, whereas the region below the wound failed to develop further. This early experiment of Malpighi demonstrated that the bark was necessary for the downward conduction of food, but not for the upward transport of water.

When a stem is girdled by the cutting away of a ring of bark, a number of different kinds of tissue are removed, such as the cortex and phloem. The question therefore arises, Does movement occur equally throughout the cortex or only in special regions of it? It was shown in 1860 by Hanstein that when a ring of extra-cambial tissues was removed from dicotyledonous plants with a single ring of collateral bundles root development was prevented.

In monocotyledonous plants with scattered bundles and dicotyledons with bicollateral bundles girdling did not result in inhibition of root formation. Hanstein arrived at the conclusion that since the removal of the phloem had resulted in inhibiting the development of roots in dicotyledons with a single ring of collateral bundles, whereas girdling had no effect on monocotyledons and dicotyledons with bicollateral bundles, the phloem formed the main channel for the downward movement of elaborated food material. The work of Czapek (1897) appeared to settle quite definitely that the phloem was the channel of transport of organic material in the higher plants. He showed, for example, that if the petioles of leaves were killed by either chloroform or steam, the removal of carbohydrates was prevented from the laminae, or if he made incisions on one side of the petiole, the removal of carbohydrates was considerably delayed in that half of the leaf. Plasmolysis of the petiolar tissues with 5 per cent potassium nitrate solution, however, did not interfere with carbohydrate transport. He therefore concluded that plasmolysis of the phloem does not interrupt the translocation of carbohydrates and that a continuous system of sieve-tubes are the necessary structural requirement for the transport of carbohydrates. As further evidence in support of the sieve-tubes being the chief organs for the translocation of carbohydrates he pointed out the well-known fact that the deposition of callus synchronizes with the cessation of translocation.

Up to 1920 the general idea that was prevalent with regard to translocation in plants was that water and dissolved salts moved up the plant in the transpiration stream through the xylem, whereas organic foods synthesized in the leaves moved downwards in the phloem. In 1920 Curtis* concluded from a number of ringing experiments that not only did organic material pass downward in the phloem from the leaves but inorganic salts moved up the plant for the most part in the same channel. His second contention, i.e. the upward passage of inorganic salts *via* the phloem, has now been shown to be untenable due in part to faulty technique and in part to misinterpretation of experimental data. Nevertheless, he has not altered his opinion on this point and still adheres to the same view. In 1922, Dixon† arrived at the diametrically opposite conclusion from Curtis, and claimed

* *Amer. J. Bot.*, 1920, **7**, 101; 1923, **10**, 361; *Ann. Bot.*, 1925, **39**, 573.
† *Pres. Address. Sect. K (Botany), Brit. Assoc.* 1922, p. 193.

that not only did inorganic salts move up the xylem, but that organic matter from above moved downwards in the wood as well. Dixon's suggestion that food material moves down the wood has now been shown to be entirely fallacious, and he himself has apparently abandoned it. One of the reasons advanced by him for the wood being the channel of transport of elaborated food material, was his dissatisfaction with the nature of the sieve-tubes with their thick, viscous, protoplasmic contents as being suitable organs which would admit of a rapid rate of flow.

Dixon's views on translocation were accepted for a few years, but the evidence now available that the phloem is the channel for the transport of organic matter is quite overwhelming. If for any reason the phloem becomes blocked or injured, translocation of food is interrupted. When the potato, for example, is attacked by a very prevalent virus disease in this country known as "leaf-roll," the leaves show an inordinately high starch-content, and it has been found that the phloem tissues in cases of severe attack are badly necrosized, and that the normal sugar of transport (sucrose) is entirely absent in any part of the diseased plants except the laminae of the leaves and a small amount in the mature tubers.

The most important investigations on translocation that have been made in the last ten years are those of Mason and Maskell, and later Mason and Phillis, on the cotton plant. These investigations were carried out in Trinidad, and represent in their wide breadth of conception and the manner in which the experiments were carried out; as well as in the interpretation of the data, one of the most brilliant series of advances that has been made in plant physiology. The enormous amount of experimental data gathered by these workers is too large to be dealt with here, except in brief outline, and the reader should consult the original memoirs, which will always remain classics of their kind.

THE TRANSLOCATION OF CARBOHYDRATES

The first investigations of Mason and Maskell* concerned themselves with the translocation of carbohydrates in the cotton plant. They investigated the total sugars, reducing sugars, and sucrose, in leaves, bark and wood of the plant over varying periods of time and the results were expressed as a percentage of the residual

* *Ann. Bot.*, 1928, **42**, 189, 571.

dry-weight (see Chapter VIII). Fig. 28 shows the series of curves obtained for one set of observations. It will be seen that the fluctuations in total sugar for leaf and bark are very similar, but that the curve for the bark sugars tends to lag behind that of the leaf sugars, whereas the total sugars from the wood shows no

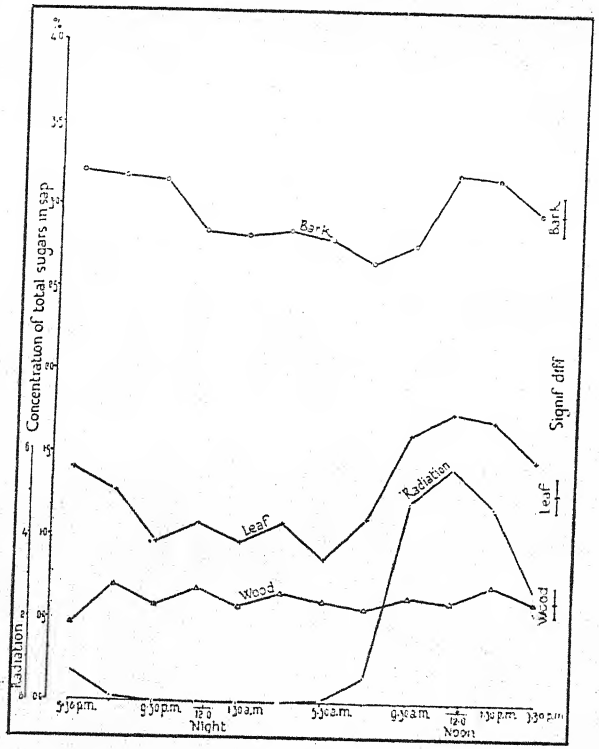


FIG. 28.—Curves obtained for the concentration of total sugars in the leaf, bark, and wood of the cotton plant. (After Mason and Maskell.)

significant variation. It was found that there was no sucrose present in the laminae of the leaves over the whole of the experimental period, and in fact it may be stated here that Mason and Maskell always found the sucrose-content of the leaves of the cotton plant to be low in sucrose. This result may have been due to their method of expressing sap from tissues which had been previously frozen to $-16°$ C. leading to a certain amount of inversion taking place.

It will be observed from the curves for these sugar concentra-

tions in the different tissues, that the concentration of reducing sugars of the wood showed no significant variation during the whole period of investigation, whereas there was a significant variation in the sucrose-content. In the bark tissues the curve for the reducing sugars shows no particular pattern, whereas that of sucrose shows marked fluctuations and follows a definite course, falling during the night and rising during the hours of light.

There are two possibilities to be considered here. In the first place sucrose may be the sugar of translocation and may be translocated in this form through the bark tissues, or, in the second place, sugar may be translocated down the outer layers of the wood, and later be moved from wood to bark. If this second possibility be correct, then the sucrose of the bark would be merely a temporary storage product.

The data were submitted to statistical treatment and the results are given below:

Correlations between Sugar Concentration in Sap of Leaf and Sugar Concentration in Sap of Bark and Wood. Diurnal Series 1

	Correlation between Concentration of Total Sugars in Leaf and Concentration of			
	Total Sugar in Bark	*Total Sugar in Wood*	*Sucrose in Bark*	*Sucrose in Wood*
1. Direct Correlation ..	+0·4626	+0·1907	+0·**5827**	+0·3652
2. Partial Correlation, Allowing for Trend with Time	+0·**7559**	+0·0109	+0·**7868**	+0·2044
	$P = 2·6\%$		$P = 4·4\%$	
3. As 1, but Leaf Values shifted 2 hours	+0·**7894**	+0·3831	+0·**9146**	+0·**6510**
	$P = 9·1\%$		$P = 6·0\%$	
4. As 2, but Leaf Values shifted 2 hours	+0·**9404**	+0·4498	+0·**9773**	+0·**6723**
	$P = 1·0\%$		$P = 0·5\%$	

Of the direct correlation coefficients that were calculated, the only one that was statistically significant was that between the total sugars in the leaf and the sucrose of the bark. Examination of the curves shows that the general drift in time of sugars in all three tissues is not the same, and by calculating the partial correlation coefficients with time eliminated, a better idea of the

drift of the sugars in these tissues is obtained. It will now be seen that the partial correlation between total sugars of the leaf and total sugars in the bark is significant, and the partial correlation between total sugars in the leaf and sucrose in the bark which was initially significant has risen to a greater value, whereas the leaf-wood correlations have fallen.

A further fact that may be observed from these curves is that sugar concentrations of the bark appear to lag behind those of the leaf. If there be a significant connection between sugars of the leaf and sugars of the bark, the correlation coefficient between them should be increased by shifting on the bark and leaf values a period (in this case two hours). It will be seen from columns 3 and 4 of the above table that such an increase is brought about. Although the correlation between leaf-wood sugars is now significant, this value is far below that of the leaf-bark value.

The significance of these results are best stated in Mason and Maskell's own words: "The curve shown for the sugar concentrations in the bark sap may thus be described in terms of a diurnal fluctuation in the leaf and superimposed on a general downward drift; the drift being due presumably to a high ratio, for the time being, of loss to gain." It is clear from these experimental results that leaf sugars are closely related to bark sugars, and the term "bark" here covers all the tissues outside the xylem and therefore includes the phloem, and that changes in the concentration of sugars in the leaf are reproduced within a few hours in the bark at a minimum distance from the leaf.

Girdling of the stem by removal of a ring of bark had a profound effect upon the translocation of carbohydrate in the stem. Above such a ring there was at first accumulation of sugars in leaf, bark and wood, whereas below the ring there was a very considerable fall in sugars of both bark and wood within a period of little more than seven hours. Evidently the fall in sugar concentration below a ring is due to the fact that the downward passage of sugar to the tissues below the ring is interrupted, whereas the ringing of the stem has not prevented the movement of sugar from leaf to bark above the ring. It was also found that organic connection between wood and bark was not necessary for normal conduction to take place, and when the bark tissues were separated from the wood by means of paraffined paper conduction took place at practically the normal rate.

The translocation of carbohydrates to the developing bolls was

also examined, and it was found that the sucrose supply to the bolls was transported four times as fast by day as by night. Moreover, concentration gradients were discovered from bark to boll, and similar concentration gradients were found from leaves to stem. Samples of bark taken from different levels of the same stems showed a concentration gradient down the stem to the roots, which resembled the movement of diffusion inasmuch as the direction of movement was from a point of high concentration to one of low concentration. It was suggested by Mason and Maskell that since the major fluctuations in the bark were due to sucrose that the bulk of the sugar in the plant travels in this form.

This work of Mason and Maskell shows beyond all reasonable doubt that the translocation of carbohydrates in the cotton plant takes place through the phloem and not through the wood, and these investigators consider that the general picture of transport of carbohydrates in the cotton plant takes place as follows: in the first place reducing sugars move along a concentration gradient from the photosynthetic cells into the sieve-tubes and are synthesized in this region into sucrose. It is considered that there is no leakage back of sucrose into the leaf cells because the cells of this tissue are relatively impermeable to this substance. In this way a considerable head of sucrose is formed in the sieve-tubes of the leaf. If this be the case, then the concentration of sucrose in the mid-rib should be higher than in the lamina of the leaf, and the concentration of reducing sugars in the lamina of the leaf should be higher than in the sieve-tubes of the leaf, and this was found to be the case.

Phillis and Mason* have analysed the saps expressed from various regions of the leaves of cotton plants, which were divided into the following parts for experimental purposes: lamina (excluding midribs), veins and petiole. They further subdivided the petiole into "outer bark" and "inner bark" and "wood." The "inner bark" was discovered to consist mainly of phloem. It was found that the diurnal fluctuations in sucrose of lamina and petiole were marked, whereas the reducing sugars showed but slight fluctuations (Fig. 29). The evidence presented shows that sucrose is the chief form in which sugars are translocated in the cotton plant. For example, it was found that sucrose is the only sugar to show well-marked and consistent

* *Ann. Bot.*, 1933, **47**, 585.

diurnal changes in both lamina and petiole, and when translocation was interrupted in any way, for example by ringing the stem, the changes in the concentration of sucrose in lamina and petiole was very much greater and more rapid than for the reducing sugars.

Phillis and Mason also found that the concentration of sucrose in the mesophyll tissues of the leaf is much less than the concentration of this sugar in the veins, yet it is from the mesophyll

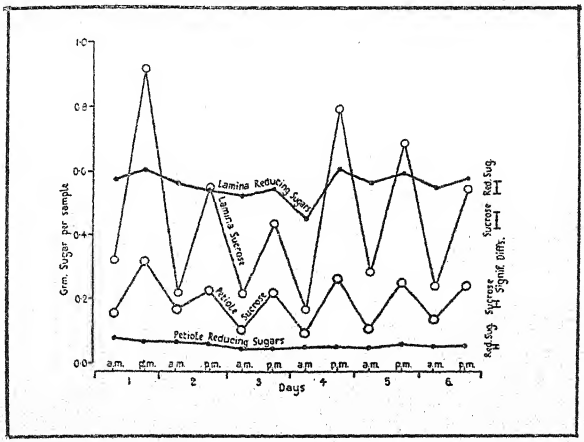


FIG. 29.—Curves showing fluctuations in reducing sugars and sucrose in the lamina and petiole of the cotton plant. (After Phillis and Mason.)

tissues that the veins receive their supply of sugar. Thus, there is a reverse gradient, and in the circumstances there must be some mechanism present whereby the phloem is able to accumulate sucrose against a steep concentration gradient. These investigators put forward the suggestion that the companion-cells and enlarged companion-like cells that surround the sieve-tubes in the fine veins of the leaf may be responsible for the accumulation of sucrose in the sieve-tubes, the function of the companion cells being to accumulate sucrose from the adjacent parenchyma and release it into the sieve-tubes.

THE TRANSLOCATION OF NITROGENOUS SUBSTANCES

The problem of translocation of nitrogenous substances in the cotton plant has proved a stubborn one to solve. It was found

by Maskell and Mason* that diurnal determinations of total nitrogen, including nitrate nitrogen, in leaves and bark, when expressed as a percentage of the wet-weight, follow a definite pattern. In the leaf the nitrogen rose by day and fell by night. In the bark, on the other hand, an accumulation of nitrogen was found during the night and early hours of the morning. This rise in the nitrogen values of the bark was followed by a fall for some hours and then a second rise. Statistical examination of the data showed that for the leaf there was a

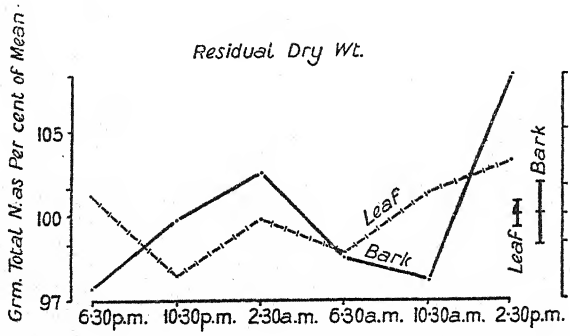


FIG. 30.—Diurnal fluctuations in total nitrogen in the leaf and bark of the cotton plant expressed as a percentage of the residual dry-weight. (After Maskell and Mason.)

significant variation of nitrogen with time, whereas in the bark the statistical data scarcely warranted the assumption that there was a variation with time of nitrogen. Nevertheless, the direct correlation coefficient between leaf total nitrogen and bark total nitrogen proved to be significant and was increased when the values were advanced a period.

Thus it would appear from the results expressed on a wet-weight basis that concentration changes in nitrogen in the leaf lead to similar changes in the concentration of diffusible substances in the bark, and on this account physical diffusion may play a part in the carriage of organic nitrogen from the leaf.

When these results, however, were expressed as a percentage of the residual dry-weight, a number of serious difficulties were encountered. Although a certain amount of evidence was found that nitrogen showed a variation in time in the leaf, this was not the case for the bark (Fig. 30). Moreover, the correlation coefficient

* *Ann. Bot.*, 1929, **43**, 205, 615; 1930, **44**, 1, 233, 657.

between leaf total nitrogen and bark total nitrogen was not statistically significant and did not increase when the leaf figures were advanced a period.

Determinations of the total nitrogen when the plants were bolling freely were also made. In the leaf a definite variation of nitrogen with time was ascertained, but no such significant variation occurred in the bark tissues. It was therefore suggested by Maskell and Mason that, when the plants were freely fruiting, the main mass of organic nitrogen was removed to the developing bolls and not translocated to the roots.

When the stems of the cotton plant were ringed, it was found that the leaves still continued to gain nitrogen. Ringing of the stem, therefore, had not interfered with the entrance of nitrogen into the leaf, showing that the translocation of nitrate from the soil must be through the wood and not the bark. This result supports the older view that inorganic nitrogen passes from the soil up the wood and not, as has been suggested by Curtis, up the phloem. Accumulation of nitrogen was found in the bark above a ring, and a small fall below the ring, but the value was not statistically significant. Lastly, it was found that when the bark was separated from the wood, nitrogen could still enter the bark, so that here again, as in the case of the translocation of carbohydrates, organic union between wood and bark is not necessary for the removal of nitrogen from leaves to roots.

When a detailed analysis was made of the different nitrogen fractions, still further difficulties and complications were discovered. The fractions estimated were ammonia N, asparagine N, amino-acid N, nitrate N, residual N and protein N. The term "residual N" was introduced to cover the crystalloid nitrogen not accounted for by the sum of the ammonia N, asparagine N, amino-acid N and nitrate N. The fraction termed "protein N" was obtained by subtracting the value of the total soluble N from the total N.

When an examination was made of the vertical gradients of these various nitrogen fractions in the stem bark, it was found that with the exceptions of the sugar gradients and possibly ammonia N, the gradients for the nitrogen fractions were negative, and the major portion of the crystalloid nitrogen gradient was due to asparagine N. For the purposes of this experiment the plants were divided into an upper and lower region and the figures obtained are shown on p. 330.

Vertical Concentration Gradients in the Bark

(Milligrams Nitrogen or Grams Sugar per 100 c.c. of Sap)

	5 p.m.	5.30 a.m.	Mean	Standard Deviation	
				Gradient	Mean Gradient
Total Cryst. N ..	−113·5	−102·9	−108·2	7·54	5·33
Organic Cryst. N	−97·1	−95·1	−96·1	7·48	5·29
Asparagine N ..	−87·2	−94·4	−90·8	7·64	5·40
Amino-acid + Residual N	−9·9	−0·7	−5·3	4·30	3·04
Nitrate N.. ..	−16·4	−9·2	−12·8	3·09	2·18
Ammonia N ..	—	+1·4	+0·7	—	—
Total Sugars ..	+1·707	+1·286	+1·497	0·257	0·182
Sucrose	+0·954	+0·851	+0·903	0·179	0·127
Reducing Sugars..	+0·753	+0·435	+0·594	0·111	0·078

It has generally been assumed that nitrogen in the plant is translocated as asparagine, and Chibnall has especially championed this view (see Chapter X), but in the cotton plant the gradients of asparagine N are negative and moreover this fraction forms the major portion of crystalloid N in the bark of the cotton plant.

In the wood, and also the leaf, the gradient of crystalloid N was found to be positive. The positive gradient in the wood was composed of nitrate N (slightly more than half), but there was also a positive gradient of organic N. A positive gradient of nitrate N was found from petiole to midrib and from midrib to leaf parenchyma, whereas the organic crystalloid N showed a positive gradient from leaf parenchyma to midrib and from midrib to petiole. This positive gradient of crystalloid N out of the leaf was almost entirely composed of residual N, although a small positive gradient of amino-acid N in the same direction was also discovered.

It will be remembered that Mason and Maskell suggested that the translocation of carbohydrate takes place by the formation in the first place of a head of reducing sugars in the leaf, which move along a concentration gradient from leaf parenchyma to sieve-tubes. In the sieve-tubes the reducing sugars are synthesized to sucrose, and as sucrose the carbohydrates are

transported down the plant. The suggestion put forward for the translocation of organic nitrogen by these investigators is that a head of residual N is formed in the leaf parenchyma. In the stem, however, a negative gradient of crystalloid nitrogen is encountered, composed in the main of asparagine N. It is therefore further suggested that there is a dynamical gradient of crystalloid N (mainly residual N) superimposed on the static gradient of crystalloid N. Thus the presence of the negative gradient of asparagine N in the stem is a phenomenon of storage.

Further work was carried out in an attempt to elucidate the method of transport of organic nitrogen in the cotton plant. For example, it was found that when transport was brought to a standstill by removing the leaves of a plant and ringing it at the base, the concentration of sugar was approximately the same at different vertical levels in the bark, but the nitrogen behaved in a different way from the sugar; the vertical gradients showed little change, and a definite negative gradient of crystalloid as well as protein nitrogen still remained. Thus zero movement of sugar is associated with zero gradient, whereas zero movement of nitrogen is associated with a negative gradient.

When the normal direction of translocation was reversed, it was discovered that the change in normal direction of movement of sugars was accompanied by a reversal in the gradient of total sugars, while there was a steepening of the original negative gradient of organic nitrogen. Maskell and Mason interpreted this result as showing a change in the nitrogen gradients by the reversal of a positive dynamic gradient of organic nitrogen originally present, superimposed on a relatively static gradient. It was further ascertained that these changes occurred mainly in the inner half of the bark, which suggests that the dynamic gradient of nitrogen is localized in the sieve-tubes, while the static gradient is principally a storage phenomenon of the ray and cortical cells.

The hypothesis put forward by Maskell and Mason to account for organic nitrogen transport in the cotton plant is similar in its general outline to their view of sugar translocation. A head of residual nitrogen is postulated in the leaf. Within the sieve-tubes all the labile forms of nitrogen, including soluble protein, take part in longitudinal movement, and the part played by each depends on the effective concentration gradient in force, but the main components of translocatory nitrogen appear to be amino-acids and residual N.

Further evidence* for the view that a storage component is masking a translocatory gradient was discovered in an ontogenetic study of the vertical distribution of concentrations in the main axis. Thus, it was found that substances which show no sign of storage in the bark, e.g. potassium, have a consistent positive gradient, whereas polysaccharides and nitrogen which accumulate steadily in the bark show a negative gradient. That asparagine N is the main storage component of nitrogen in the bark was fully confirmed, whereas residual N, on the other hand, was found to maintain a consistent positive gradient and possibly represents the mobile constituent of nitrogen.

THE TRANSLOCATION OF MINERAL SALTS

The traditional view of the translocation of mineral salts in the higher green plants has always been that the salts are absorbed from the soil solution by the root hairs and carried upwards to the various developing and synthetic regions *via* the xylem in the transpiration stream. This view of the upward path of mineral salts has been fully confirmed by a number of recent investigations, notably those of H. F. Clements, working on a variety of fruit trees, and Mason and Maskell† on the cotton plant. The older view was, however, challenged by Curtis, who claimed to have shown that not only is organic matter translocated from the synthetic centres of the plant in the phloem, but that inorganic salts are also transported from the soil in the phloem and in the wood.

Curtis arrived at this conclusion by using two different types of procedure. He found that the girdling of stems of various plants interfered with the upward transfer of solutes. This is no doubt correct, but it is not logical to assume that therefore mineral constituents must pass up the phloem as well. To what extent the upward translocation of inorganic salts from the soil will be interrupted by ringing of the stem, will depend on the anatomical structure of the stem. In the grape, for example, ringing of the stem has no effect on the upward transfer of salts from the soil, whereas in *Prunus americana*, removal of a ring of bark severely handicaps the upward movement of solutes from the soil, owing to the anatomical structure of the stem of this plant.

* *Ann. Bot.*, 1934, **48**, 119, 315.

† *Ann. Bot.*, 1931, **45**, 125.

Curtis* also studied the effect of chilling various parts of a plant and found that translocation was interrupted. He found that when the petioles of *Phaseolus vulgaris* were chilled between 1° and 4° C. the upward passage of inorganic material was interfered with. Curtis' interpretation of this result as showing that inorganic solutes must pass up the phloem is again illogical, for not only will the processes taking place in the living cells be interfered with, but the rate of water movement through the non-living tracheal system will also be interrupted, for such factors as the viscosity of the water will be increased with lowering of the temperature and this will cause resistance to flow through the small tracheal capillaries. In any case the xylem sap is not pure water, but contains salts, sugars, organic acids and soluble nitrogenous compounds, which will also tend to increase the resistance to flow at ordinary temperatures as well as increase the degree of viscosity change following a fall in temperature.

It has been shown by Mason and Maskell in a preliminary investigation of the movements of potassium, phosphorus and calcium in the cotton plant, that the upward movement of these elements is through the xylem. For example, when a stem was ringed there was accumulation of these elements above the ring and diminution below the ring. These results show that the ash constituents must pass up the wood to the foliage and are re-exported from these regions *via* the bark. They could, however, find no evidence that calcium is re-exported from the leaves. They also examined the manner in which these ash constituents were carried to the bolls, and found that while calcium was possibly translocated to the developing fruits *via* the xylem, phosphorus and potassium were transported to these regions from the leaves through the phloem.

The immobility of calcium discovered by this work is a difficult problem to explain. One reason for its immobility appears to be the inability of this element to move in the phloem. In the cotton plant calcium oxalate is abundant in the ray cells of the phloem, but no definite evidence could be found for its presence in the sieve-tubes. The plant requires calcium throughout its growing period, whereas phosphorus, potassium and nitrogen are only required in the early stages of growth, and stoppage of the supply of these elements after a certain period does not interfere with normal development. It is suggested by Mason and Maskell that

* *Amer. J. Bot.*, 1929, **16**, 154.

calcium may gain entry to all cells *via* the transpiration stream, but once it has entered the cells it may be precipitated or combined with tissue material in such a manner that few calcium ions are left in solution. Certainly one way in which calcium is fixed in the leaf cells is in the form of the calcium salt of the nitrogen-free lipin, phosphatidic acid.

THE MECHANISM OF SOLUTE MOVEMENT IN THE PHLOEM

The precise mechanism of the translocation of solutes in the phloem is not known. A number of suggestions, however, have been put forward to account for this movement in these tissues. Until recently it was generally assumed that this movement is one of physical diffusion. Thus sugars are synthesized in the leaves, and from this initial source diffuse away to the place of utilization or storage as the case may be. There are considerable difficulties in accepting this simple view of translocation. The chief obstacle in the way of acceptance is that of speed. Dixon calculated that if sugar entered a potato tuber through the phloem in a 10 per cent solution it would be necessary for it to move at the rate of 50 cm. per hour. Actually the concentration is very much smaller than this, and somewhere in the neighbourhood of 4 to 5 per cent. Similarly it has been shown by Mason and Maskell that in the cotton plant the movement of sugar through the phloem is 40,000 times as great as the diffusion-constant in a 2 per cent solution of sucrose in water, and almost identical with the diffusion-constant for molecules the size of sucrose molecules diffusing in air.

It was suggested by de Vries that the protoplasmic streaming which has been observed in many plant cells may adequately account for the rapid rate of movement of solutes through the phloem. This hypothesis has been revived by Curtis. The main objection to this view is that protoplasmic streaming has not been observed in mature sieve-tubes. Curtis considers that injury in the course of examination may have brought this streaming to an end in mature sieve-tubes. But this is an assumption that has not been experimentally verified, and until such time as it has been proved that injury does put an end to this movement, this hypothesis cannot very well be accepted.

Two further theories of solute movement in the phloem remain to be considered, and it may be said at once that there are serious objections to the complete acceptance of either.

Münch* has put forward the view that the protoplast of the sieve-tubes is very permeable to mass movements of water and that there is a mass movement of water through the sieve-tubes and the plasmodesmal connections. The Münch hypothesis is best illustrated by Fig. 31. In A there is a solution of high osmotic concentration surrounded by an osmotic membrane, while in tube T and in B, which is also an osmotic membrane, there is water. Both membranes A and B are immersed in water. Water will diffuse into A across the membrane on account of the steep diffusion gradient across this membrane. As a result a pressure will be developed throughout the system, and since there are no

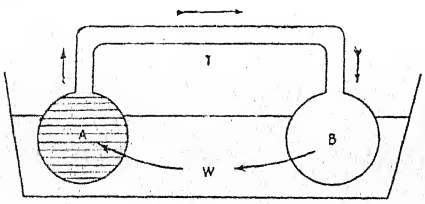


FIG. 31.—Diagrammatic illustration of the principle of osmotic flow: A is an osmotic membrane with a high concentration; B an osmotic membrane with low concentration connected by an open tube T. The feathered arrows show the direction of flow of the solution from the cell or part of cell with high concentration to that with low. The direction of flow of water is shown by the plain arrows. (After Münch. From Curtis, *Translocation in Plants*.)

solutes in B, or if their concentration be low, there will be no resistance or a very small resistance to the diffusion of water through membrane B to the external water again. A mass flow of solution from A to B will take place, and with rise in the concentration of the solution in B, there will be a rise in the resistance to the diffusion of water from B to the external water, and the pressure in the whole system will increase. Now, if the solute be removed in some way when it arrives at B, water will continue to pass out readily through the membrane and solution will continue to pass from A to B. Translocation in the potato would be explained on the Münch hypothesis by assuming that a solution of sugar would be moved into the tuber by mass movement through the phloem. Once arrived in the tuber, the sugar would be deposited, presumably as insoluble starch, and the water would find its way back into the xylem and be returned to the plant through this channel.

* *Ber. deut. bot. Ges.*, 1926, **44**, 68; 1927, **45**, 340; 1932, **50**, 407.

Münch's view of translocation by mass movement does not allow of a two-directional movement taking place simultaneously through the phloem, and there is a considerable amount of evidence available to show that there is such dual directional flow of solutes in this tissue. Further objections to the Münch hypothesis are that the proposed mechanism does not allow for the movement of specific substances to specific cells, for they must all move together, and the pressure gradients are not sufficiently great to allow of a flow of solution through the conducting tissues into the receiving cells and exudation of water from the receiving cells.

Crafts* has put forward a somewhat similar hypothesis to that of Münch to account for translocation down the phloem. The actual flow through the sieve-tubes is considered to take place by the same mechanism as that proposed by Münch, namely, by means of mass movement of the solutes through the sieve-tubes, but Crafts has suggested that not only does flow take place through the lumen of the sieve-tubes, but that it also occurs freely across all side and cross walls and is not restricted to the pores of the sieve-plates. Nevertheless, the objections that have been raised against Münch's hypothesis apply with equal force here, and in any case the claim that there is low resistance to flow through the walls of the sieve-tubes has yet to be substantiated, nor has the claim that the protoplasm of the sieve-tubes is completely permeable been made out.

All that can be said at the present time about the mechanism of translocation of solutes through the phloem is that we know quite definitely that solutes are able to pass through this tissue at a high rate, but how the plant is able to bring about this high speed of transport has yet to be determined.

* *Plant Physiol.*, 1931, **6**, 1; 1932, **7**, 183; 1933, **8**, 81.

CHAPTER XIII

RESPIRATION

When a machine is required to do work it must be supplied with energy. In the same way the living organism requires a continuous supply of energy to enable it to carry out its vital activities. This energy is obtained by the breaking down of complex substances into simpler ones. Thus carbohydrates and fats are oxidized in the living cell to carbon dioxide and water:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$$

and the energy released in this process is used for work by the living organism. The carbon dioxide and water obtained when a hexose, for example, is oxidized in the cell, are end products of a complex series of chemical changes.

The term *respiration* has been given to the process of gaseous exchange whereby oxygen is absorbed from the atmosphere and carbon dioxide is evolved when organic matter is broken down in the cell with consequent release of energy. Thus, respiration is the direct opposite of assimilation, since in the latter process carbon dioxide and water, in the presence of light and chlorophyll, are built up into carbohydrates which are potential stores of energy. Respiration, therefore, is sometimes spoken of as *dissimilation*. The term respiration, however, is usually confined to the direct effect of oxygen on the oxidative processes of the cell, i.e. it is *oxidative dissimilation*, whereas the term *fermentation* is used for those processes in which the direct effect of oxygen does not enter into the matter. In the fermentation of hexose by Saccharomyces, carbon dioxide and ethyl alcohol are the main end products of the reaction.

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$$

while butyric acid, carbon dioxide and hydrogen are obtained by the action of *Bacillus butyricus* on hexose

$$C_6H_{12}O_6 = CH_3 . CH_2 . CH_2 . COOH + 2CO_2 + 2H_2$$

and *Bacterium lactis acidi* gives lactic acid from glucose and galactose

$$C_6H_{12}O_6 = 2CH_3 . CHOH . COOH$$

One of the differences between the two processes lies in the differences in the amount of energy released. In alcoholic fermentation 57 calories of heat are evolved for every gram-molecule of hexose fermented, whereas in ordinary respiration 674 calories of heat are evolved for every gram-molecule of hexose oxidized.

It was as far back as 1779 that Ingen-Housz demonstrated that green plants, like animals, respire. For the higher green plants oxygen is a primary necessity for the normal functioning of the living tissues, while other plants, such as the lactic bacteria, are intolerant of oxygen and die in its presence. Others again are able to tide over a period without oxygen. Various types of respiration have therefore been distinguished, such as *aerobic* (oxygen necessary), *anaerobic* (oxygen not necessary) and *facultative anaerobic*.

Throughout the life of the plant, respiration is a continuous process and never ceases in any living cell. It has been found that plants which normally respire aerobically, fail to grow normally in the absence of oxygen and exhibit a lack of response to stimuli and a general slackening of activity.

It is a difficult matter to demonstrate that green plants are continually respiring by day as well as by night, since in the presence of light the chlorophyllous cells use the carbon dioxide of respiration for photosynthesis. There is, however, indirect evidence to show that respiration is unceasing in the living cells of green plants, for in actively assimilating cells protoplasmic movement and growth may be observed, and these phenomena could not take place in the absence of respiration.

From photosynthesis there is an actual gain in dry-weight in the green plant, whereas there is a loss of dry-weight from respiration. The difference between total assimilation and total respiration represents the amount of material available for new growth. Autotrophic plants grown in continuous darkness, and heterotrophic plants in the absence of sufficient nutritive material, show a diminution of dry-weight. This decrease in dry-weight from respiration is best shown with seedlings. Some figures obtained by Boussingault are given on p. 339.

As long ago as 1840 it was shown by Hess that, when a chemical reaction between two substances took place under certain definite conditions and in definite amounts, the same amount of heat was always evolved, provided that the final products of the

reaction were the same in each case, and further, that the amount of heat given out was quite independent of the rate of combustion and the number of intermediate steps.

The formation of ammonium chloride, for instance, by the combination of one gram-molecule of gaseous ammonia with one gram-molecule of gaseous hydrochloric acid, liberates 42,100 calories of heat, while a solution of a gram-molecule of ammonium

Material	*Dry-Weight of Seeds Grams*	*Dry-Weight of Seedlings Grams*	*Loss Grams*
46 wheat grains ..	1·665	0·713	0·952
10 pea seeds	2·237	1·076	1·161

chloride in water (a large excess of water must be used) is accompanied by the absorption of 3,900 calories of heat:

$$NH_3\ (gas) + HCl\ (gas) = +42{,}100 \text{ calories}$$
$$NH_4Cl + H_2O = -\ 3{,}900 \text{ calories}$$
$$38{,}200 \text{ calories}$$

Another method of preparing ammonium chloride is to dissolve ammonia in water, and hydrochloric acid gas in water, and then add the two solutions together, thus:

NH_3 (gas) + water	= + 8,400 calories
HCl (gas) + water	= + 17,300 calories
HCl (solution) + NH_3 (solution)	= + 12,300 calories
Total	38,000 calories

Within the limits of experimental error, the same amount of heat has been evolved in these two methods of preparing ammonium chloride.

In the complete physiological combustion of carbohydrate or fat in respiration to carbon dioxide and water, Hess's law applies equally as in the case of combustion of carbohydrate or fat in oxygen. The rate of the reaction in the cell is slow and a number of intermediate steps are involved before the final products, carbon dioxide and water, are obtained, but the amount of heat is the same as if these substances were burnt in oxygen.

The combustion of 12 grams of carbon gives rise to 94,800 calories of heat, whereas the combustion of 2 grams of hydrogen yields 69,000 calories, or 1 gram of hydrogen on combustion gives rise to 34,500 calories and 1 gram of carbon to 7,900 calories. Hydrogen, therefore, gram for gram, evolves a far greater amount of heat than carbon. Consequently a compound rich in hydrogen will possess a greater calorific value than a compound rich in carbon. Since oxygen itself possesses no calorific value, the presence of a large amount of oxygen in a compound will reduce its calorific value. Fats, therefore, which possess a high hydrogen and low oxygen content, have a greater calorific value than carbo-

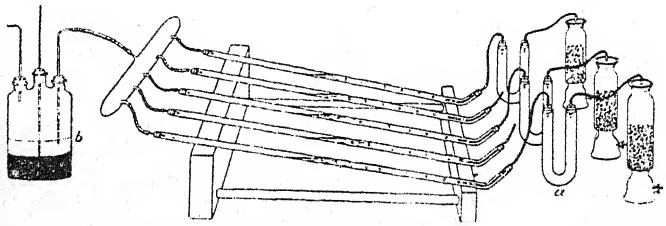


FIG. 32.—Apparatus for measuring carbon dioxide evolved in respiration. (After Pettenkoffer. From Palladin *Plant Physiology*.)

hydrates which possess a high oxygen content. A few figures of the heat of combustion of some cell substances are given below:

1 gram of fat	=	9·1 calories
1 gram of alcohol	=	7·1 calories
1 gram of protein	=	5·8 calories
1 gram of carbohydrate	=	4·1 calories

EXPERIMENTAL METHODS

For the quantitative study of respiration of plant tissues it is necessary to determine either the amount of carbon dioxide evolved in a given time or the amount of oxygen absorbed. A number of different types of apparatus have been devised for this purpose.

To determine the amount of carbon dioxide evolved in respiration, the apparatus shown in Fig. 32 may be used. Air, freed from carbon dioxide by passage through a tower filled with soda-lime, and then passed through baryta-water, which will remain clear if the soda-lime tower is acting efficiently, is allowed to flow slowly

through the chamber containing the respiring tissue (*a*, Fig. 32). The air issuing from the respiratory chamber will be mixed with the carbon dioxide of respiration, and is next passed through a Pettenkofer-tube filled with a known volume of a standard solution of baryta. The rate of flow must be so regulated that the bubbles pass singly and do not fuse. The carbon dioxide is absorbed by the baryta solution and barium carbonate precipitated. After a given time, the air stream is turned into a second Pettenkofer-tube, and the solution from the first is removed and titrated against either oxalic or hydrochloric acid. The amount of carbon dioxide given off in unit time can then be calculated.

To measure the amount of oxygen absorbed and carbon dioxide evolved in respiration, the respiring material is enclosed in a vessel containing a gas mixture of known composition. After a given period has elapsed, the amount of carbon dioxide is estimated by absorbing it with potassium hydroxide and noting the reduction in volume of the gas mixture at constant pressure. The reduction in the amount of oxygen is determined with an alkaline solution of pyrogallol. For exact determinations, in cases where there is sufficient volume of gas for this purpose, changes in the concentration of oxygen and carbon dioxide may be readily determined with Haldane's apparatus.

RESPIRATORY QUOTIENT

It was first observed by de Saussure that there is a correlation between the oxygen absorbed and the carbon dioxide evolved by a plant. This ratio of carbon dioxide to oxygen (CO_2/O_2) is termed the *respiratory quotient*. The respiratory quotient is variable in different plants, and also for the same plant at different times in its life-history, and the value is also affected by various conditioning factors.

There is an intimate relationship between the value of the respiratory quotient and the nature of the substrate used in respiration as well as the nature of the respiratory process. When carbohydrates are being used in respiration the value is in the neighbourhood of unity, provided that carbohydrate is completely broken down to carbon dioxide and water. On the other hand, when fats are the substrate for respiration, the value of the quotient is less than unity, since a large amount of oxygen

must be used in the oxidation of fat to carbon dioxide and water. This is made clear by the equations given below:

$$(1)\ C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O \text{ (Carbohydrate)}$$
$$(2)\ 2C_{51}H_{98}O_6 + 145O_2 = 102CO_2 + 98H_2O \text{ (Fat)}$$

It has been shown by de Boer,* working with the fungus Phycomyces, that the respiratory quotient varies with the nature of the substrate upon which it is grown. When a linseed substrate (i.e. a fatty substrate) was used, the intensity of the respiration was greater than when a carbohydrate substrate was used, and the respiratory quotient lay between 0·65–0·75, whereas when the fungus was grown on a bread substrate (i.e. a carbohydrate substrate) the value lay between 1·0–1·2.

The respiratory quotient also varies with the concentration of the substrate. Some values obtained by Puriewitsch† for Aspergillus grown on different concentrations of cane sugar are given below:

Concentration of Medium	1	5	10	20	25 per cent
Respiratory Quotient	0·85	0·96	1·04	0·93	0·75

When substances rich in oxygen, such as organic acids, are burnt in respiration there is a considerable increase in the quotient. Thus with oxalic acid, the value is 4:

$$2\,(COOH)_2 + O_2 = 4CO_2 + 2H_2O$$
$$\frac{CO_2}{O_2} = \frac{4}{1} = 4$$

while with tartaric acid the value is 1·6

$$2COOH—CHOH—CHOH—COOH + 5O_2 = 8CO_2 + 6H_2O$$
$$\frac{CO_2}{O_2} = \frac{8}{5} = 1{\cdot}6$$

In general, when carbohydrates form the main substrate for respiration, the end products of the reaction are carbon dioxide

* *Rec. trav. bot. Néerl.*, 1928, **25**, 117. † *Jahrb. f. wiss. Bot.*, 1900, **35**, 573.

and water; but in certain cases, such as in succulents, combustion is incomplete, and ends with the formation of organic acids. The Cactaceae form isomalic acid, the Crassulaceae malic acid and the Mesembryanthemaceae oxalic acid. The massive somatic construction of these plants with their large mass of parenchymatous tissue may account for the formation of organic acids as the end products of respiration by making the movement of gases a slow and difficult process, with the result that respiration stops at the organic acid stage. These acids are later used for photosynthesis.

A number of micro-organisms, fungi and bacteria, produce organic acids as products of respiration from carbohydrate fuel. The bacteria produce monobasic acids, such as lactic, acetic and butyric acids, whereas the fungi produce polybasic acids such as fumaric, citric and oxalic acid. The investigations of Wehmer carried out in 1891 showed that *Aspergillus niger* produces oxalic acid in large amounts as a respiration product, and he found that not only is oxalic acid formed when the respiratory substrate is glucose, but it is also produced from the salts of various organic acids, such as tartaric, malic and citric acids. This formation of oxalic acid by *A. niger* is quite independent of the supply of oxygen, but temperature is an important factor; the higher the temperature, the greater the amount of oxalic acid formed.

According to Langdon,* and also Langdon and Gailey, carbon monoxide is a product of respiration of the pneumatocysts of the Laminarian, *Nereocystis Luetkeana*. The bladders of this plant were found to contain an atmosphere of nitrogen, oxygen and carbon monoxide, but no carbon dioxide. Over a thousand of these floats were analysed and the carbon monoxide content was found to vary between 1 and 12 per cent by volume, while the oxygen varied between 15 and 25 per cent. Carbon monoxide was only formed in the presence of oxygen; if oxygen were replaced by nitrogen or hydrogen the production of the gas ceased. The formation of carbon monoxide was found to continue by day and by night, and it was not produced when autolysis was allowed to set in by grinding up the tissues. The only conclusion that can be drawn from these facts is that the carbon monoxide must be a product of respiration.

* *J. Amer. Chem. Soc.*, 1917, **39**, 149; *Bot. Gaz.*, 1920, **70**, 230.

HEAT OF RESPIRATION

The production of heat through respiratory activity is readily demonstrated. Bulked leaves and lawn mowings quickly develop a high temperature. Molisch* has determined the heat of respiration in the massed leaves of *Carpinus Betulus*. He ascertained that the leaves reached a temperature of 51° C. in 15 hours; a fall then occurred, and at the end of 48 hours the temperature was 34° C. After 104 hours a secondary maximum 47° C. was attained, and the temperature then fell to 31° C. after 180 hours. The first maximum was found to be a true expression of the heat of respiration of the leaves, while the second was discovered to be due to bacterial activity.

Peirce† has investigated the heat of respiration of germinating pea seeds: 75 grams of seeds were placed in a Dewer's flask and the temperature ascertained; the heat capacity of the flask was also calculated, as well as the loss due to radiation. The heat production per minute was also ascertained for seedlings of different ages. With swollen seeds 9 calories per minute were evolved, seedlings with roots 5 mm. long evolved 125 calories per minute. When the cotyledons had died, the heat of respiration dropped to 22 calories per minute; and finally when the cotyledons had fallen off, the heat evolved per minute fell to 6 calories. In other words the heat of respiration fell with increase in age of the seedlings.

Bonnier attempted to determine the amount of heat evolved as well as the amount of carbon dioxide produced by germinating seeds. A special calorimeter was used for this purpose, consisting of a double-walled test-tube which was fitted with a stopper and a mercury manometer. The outer jacket was completely filled with mercury and the germinating seeds were placed within the inner jacket. As the seeds respired, heat was produced, and the mercury in the manometer expanded. It was therefore a simple matter to calculate the amount of heat as well as the temperature. The amount of carbon dioxide evolved was also estimated. There is one serious criticism of this investigation that should be stated here, namely, that the gradual accumulation of carbon dioxide within the inner vessel will eventually depress the rate of respiration of the seedlings very considerably. Using barley grain, Bonnier allowed germination to proceed until the first leafy stem began to appear, and obtained the following values:

* *Bot. Zeit.*, 1908, **66**, 211.

† *Bot. Gaz.*, 1912, **53**, 89.

Stage of Development	*Heat Production per Minute*		*Respiratory Quotient*
	Calories Found Gram-Calories	*Calories Calculated Gram-Calories*	
Soaked seeds	5	3	1·0
Roots beginning.. ..	62	45	0·65
Roots 3 mm. long ..	40	31	0·80
Germination over ..	15	12	0·95
Leafy stem developed ..	0	3	1·0

The calculated values for the heat evolved were obtained from the rates of carbon dioxide evolution and oxygen absorption. From the differences between the observed and calculated values it is obvious that the combustion of carbohydrate does not cover all the katabolic processes of the cell. There must be some other process or processes involved which are exothermic, and it may well be due to these that the above differences were obtained. Among such reactions, the inversion of starch by amylase activity and other hydrolytic activities of enzymes must be included. It should be noticed that the heat evolved is at a maximum when the respiratory quotient is at a minimum.

As a general rule, the internal temperature of the living plant is little above that of the surrounding atmosphere. There are three periods in the life of the plant when there is an appreciable rise in temperature. The first period is at germination, the second at the opening of the buds of deciduous trees and the third at flower formation. A temperature of 49° C. has been observed in the spathe of some of the Aroideae, when that of the surrounding atmosphere was only 19° C.

Since respiration is the source of energy for the plant, the active evolution of heat represents an excess of energy and is therefore a waste product. Excess of heat is not a true criterion of the amount or intensity of respiration, but merely reveals the inefficiency of the plant in this respect.

INTENSITY OF RESPIRATION

There is at present no accurate method of expressing the intensity of respiration. The true measure of intensity of respiration is the rate at which energy is set free by the breakdown of the respira-

tory substrate and upon the final products formed. In actual practice the amount of carbon dioxide evolved, or sometimes the amount of oxygen absorbed, has been employed by different workers as a measure of intensity of respiration; the data being referred to either fresh- or dry-weight of the particular tissue under investigation. But as the various steps concerned in the breakdown of the respiratory substrate are still unknown or in a state of controversy, it is not surprising that there is a certain amount of confusion over this question of intensity of respiration.

In general terms it may be stated that the respiration of succulents and shade-loving plants is low, while the respiration of some of the fungi may exceed that of warm-blooded animals. Germinating seeds, developing flowers and buds all show a high rate of respiration. Some values obtained as long ago as 1851 by Garreau are given below:

Temperature 16° C.

	Fresh-Weight of Seeds	*Dry-Weight of Seeds*	*CO_2 in 24 Hours*	*CO_2 per gm. Dry-Weight*
	gm.	*gm.*	*c.c.*	*c.c.*
Papaver somniferum ..	5·8	0·45	55	122
Sinapis nigra	8·5	0·55	32	58
Valerianella olitoria ..	4·0	0·20	25	125

The following values were obtained for buds:

Temperature 16° C.

	Fresh-Weight of Buds	*Dry-Weight of Buds*	*CO_2 in 24 Hours*	*CO_2 per gm. Dry-Weight*
	gm.	*gm.*	*c.c.*	*c.c.*
Syringa vulgaris ..	9·0	2·0	70	35
Ribes nigrum ..	7·0	1·25	60	48
Tilia europaea ..	4·0	0·70	46	66

Bonnier and Mangin considered that the plant taken as a whole shows two maxima in its seasonal development, the first on the unfolding of its leaf-buds and the second on the opening of the flower buds. According to Nicolas,* who has made a

* *Rev. gen. Bot.*, 1918, **30**, 209.

study of the respiration of the vegetative parts of annuals, biennials and perennials, the leaves and portions of the stems of the same branch vary in the intensity of their respiration with age; those at the apical regions having a more intense respiration than portions from the lower parts of the plant. In some cases the intensity of respiration was found to be seven times greater in the higher regions than in the lower portions. It has been shown that the intensity of respiration of young leaves is greater than old ones, and in all cases the actively growing parts of plants show an increase in respiration over non-growing parts.

A full investigation of the respiration of *Helianthus annuus* has been made by Kidd, West and G. E. Briggs.* The respiration of a representative plant of a crop was determined at frequent intervals at constant temperature. From the results thus obtained it was possible to estimate the respiration of a mean plant of the crop at the recorded fluctuating temperatures under field conditions, and a measure was obtained of the loss of dry-weight of the plant. The effect of age on respiration was also obtained from these values. It was further shown that there is a group of factors within the plant which will affect the particular process or group of processes. Each such group was termed by these investigators the "internal factor" for that process.

The following factors were found to be important in their effect on the rate of respiration per unit of dry-weight of the plant: (i) the concentration of respirable substrate, (ii) the effective amount of respirable cell-matter per unit of dry-weight (this is termed the "internal factor" for respiration), (iii) the concentration of oxygen and (iv) the temperature.

The internal factor for respiration is most readily determined when the other factors are not conditioning the respiration rate. The respiration expressed as per gram weight of dry matter per hour, when measured with respiration material in excess, with the external oxygen concentration at that of the atmosphere and at a temperature of 10° C., was termed the *respiratory index*. It was found that there was a continuous falling off in the respiration rate with advance of age as measured by the respiratory index, and this applied to stem, leaves and flowers. The respiratory index of the stem apex was also found to decrease with age, indicating that the respiratory index of the meristematic tissues falls with age. This fall in the respiratory index with age in the

* *Proc. Roy. Soc.* (*Lond.*), 1921, 92B, 368.

meristematic tissues and leaves shows that the fall for the whole plant is not due to increase in xylem, sclerenchyma and other mechanical tissue.

The intensity of respiration in certain cases appears to be dependent on the presence or absence of pigments in the leaf. Nicolas* found that leaves containing anthocyanin pigment, either in youth form or as a permanent character, absorbed more oxygen and showed an increased rate of respiration compared with normal green leaves.

According to F. F. Blackman, there are two types of respiration always proceeding simultaneously in the living cell. The first consists of an oxidation of fat or carbohydrate to carbon dioxide and water. To this type of oxidation he has given the name *floating respiration*, and to the second he has applied the term *protoplasmic respiration*, which is supposed to be the necessary minimum for the maintenance of life. Further there are two stages in the process, of which the first is an anaerobic splitting of carbohydrate into carbon dioxide and some easily oxidizable substance, and the second stage lies in the oxidation of this second substance by the oxygen of the air. Kidd has shown that the narcotic action of carbon dioxide on respiration is exerted only on the first process. Thus, fundamentally considered, aerobic and anaerobic respiration have the same genesis and differ only in their later stages. This was a view that was put forward many years ago by Pfeffer, yet it is an experimental fact that yeast does not ferment any more sugar to carbon dioxide and alcohol in the presence of oxygen than in its absence. On the other hand, it must be remembered that yeast has been a cultivated organism for many generations, and may on this account behave in an indifferent manner to the presence or absence of oxygen. The nature of aerobic respiration, however, is considered in further detail in a later section.

FACTORS AFFECTING RESPIRATION

There are a number of external and internal factors which influence the rate and intensity of respiration. The more important of these are (i) amount of respirable material, (ii) concentration of oxygen in the air, (iii) water, (iv) concentration of carbon dioxide in the air, (v) acidity, (vi) salts, (vii) temperature and (viii) light.

* *Compt. Rend.*, 1918, **167**, 131.

Respirable Material.—Respiration in the cell is ultimately dependent upon the food supplies of the cell, since these form the substrate for respiration. Kosinski* grew *Aspergillus niger* in a flask on a nutrient medium containing glucose, and determined the respiration rate by carbon dioxide output. By means of a siphon arrangement he was able to draw off the glucose medium at will and replace it by water. The curve obtained for his results is

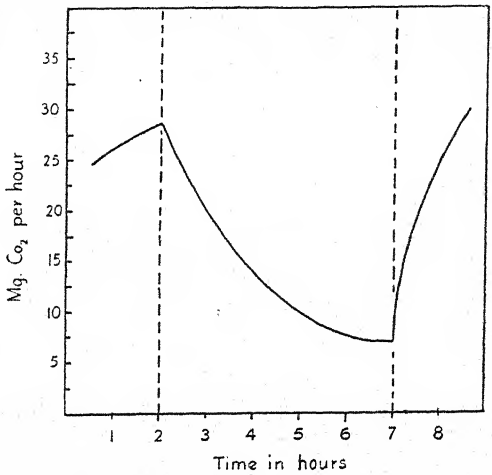


FIG. 33.—Change in respiratory rate, measured in milligrams of carbon dioxide evolved per hour, of *Aspergillus niger*, according to the presence of glucose. (After Kosinski. From Bayliss, *Principles of General Physiology*.)

shown in Fig. 33. As soon as the glucose was removed and replaced by water there was a rapid fall in the rate of respiration, which, however, showed no signs of falling to zero. On the readdition of glucose, the respiration rate rose once more.

Carbohydrates are an extremely important substrate for respiration. Etiolated leaves respire at a lower rate than normal green leaves owing to lack of carbohydrate. Palladin has shown that 100 gm. of etiolated leaves of *Vicia Faba* at room temperature evolved carbon dioxide at the rates of 102·8, 95·9 and 70·2 mg. per hour for three successive hours. The average rate was 89·6 mg. per hour. The same leaves when floated upon a solution of cane sugar in the dark for two days, showed an increase of sugar content, while the protein content was not affected. The rate of

* *Jahrb. f. wiss. Bot.*, 1902, **37**, 137.

evolution of carbon dioxide was now found to be 152·6, 147·5, 146·8 and 144·5 mg. per hour respectively for four successive hours, with an average rate of 147·8 mg. per hour. A longer treatment of the leaves than two days with cane sugar showed a higher carbohydrate content, but the rate of respiration was not increased. Even treatment for forty hours produced no greater evolution of carbon dioxide than treatment for four hours. It follows from these results that although carbohydrate supply is necessary for respiration, there is no correlation between amount of carbohydrate and the rate of respiration.

Palladin endeavoured to carry out a parallel series of determinations of the amount of carbon dioxide evolved and the amount of indigestible proteins (proteins not digested by gastric juice) which are present in wheat seedlings germinated in the dark. The rate of elimination of carbon dioxide was stated to be proportional in the intermediate stages of growth to the amount of protein in the seedling. In the later stages of growth, the respiratory rate was found to decrease on account of diminishing supplies of carbohydrate, but the quantity of indigestible proteins was found to increase in amount. Palladin claimed that it is only the proteins which are not constituents of the protoplasm which decrease through respiratory activity, and that the amount of living protoplasm can thus be approximated in terms of the amount of indigestible proteins. It is, however, extremely doubtful if much reliance can be placed upon these results as the experimental technique is open to criticism.

It was found by Palladin that in wheat grains germinated at a temperature of 20–21° C., the ratio of hourly carbon dioxide production to the amount of nitrogen in the pepsin insoluble fraction of protein, i.e. the ratio CO_2/N, was as follows in a germination period of nine days:

Days	CO_2/N
4	1·06
6	1·05
7	1·18
9	1·15

In other words, the value of the ratio CO_2/N is a constant for different plants at a given temperature and in the presence of adequate supplies of carbohydrate. For example, in lupin seedlings the value of the ratio was found to be 1·12, and in the etiolated leaves of *Vicia Faba*, when treated with a solution of

cane sugar, 1·10. According to Palladin, $CO_2/N = 1 \cdot 1$ for a temperature of 19–20° C.

The respiration rate of sweet potatoes has been found to be high compared with ordinary varieties by Müller-Thurgau and Schneider-Orelli.* The respiratory rate is dependent upon sugar supplies, for, in the autumn, when the stock of sugar is low, the respiratory rate is also low, but with increase in age and accumulation of sugar the rate of respiration rises again. Spoehr† claims that in the absence of hexoses polysaccharides are consumed in respiration, and that pentosan formation in these plants is possibly in some way connected with this method of respiration.

Oxygen.—For the proper functioning of aerobic respiration in the green plant an adequate supply of oxygen is necessary. The amount needed is independent within wide limits of the percentage of oxygen in the air. The partial pressure of the oxygen may be reduced or increased considerably without influencing the rate of respiration. It has been found that if the pressure of pure oxygen be raised to 2 to 5 atmospheres it brings about a marked increase in the rate of respiration and that this increase is followed by a decline until the death of the plant. Death under these conditions is not due to an excessive rate of respiration, but to excess of oxygen. When the oxygen supply is reduced, the respiration rate is not at first affected, and it is only when the supply of oxygen falls below 2 per cent that the respiration rate shows a fall.

Carbon Dioxide.—Carbon dioxide when present in high concentration acts as a depressant upon the rate of respiration. Excess of carbon dioxide is quickly removed in the assimilating leaf in the presence of light, and likewise should any accumulation of carbon dioxide occur during the period of darkness, it will be decomposed on the advent of light by the activation of the assimilating mechanism. The presence of intercellular spaces in the leaf also prevents the accumulation of any high concentration of this gas, and even in the interior of the cells oxygen is present in sufficient concentration to prevent the toxic effects of high carbon dioxide concentrations. In the leaf, the stomata open in darkness to get rid of excess of carbon dioxide. That the supply of oxygen is sufficient within normal cells to prevent the toxic action of carbon dioxide has been demonstrated by Celakowski, who showed that in the plasmodium of a myxomycete which had

* *Flora*, 1910, **101**, 309. † *Carnegie Inst. Pub.*, 1919, 287.

ingested the hairs of Tradescantia, the protoplasmic streaming which is a feature of these hairs, and which ceases in the presence of a high concentration of carbon dioxide, continued within the plasmodium.

The germination of seeds is retarded or inhibited in the presence of high concentrations of carbon dioxide. This inhibitory effect may continue after the seeds have been removed from the atmosphere of carbon dioxide. In *Brassica alba*, the inhibitory effect of carbon dioxide on germination is so great, that the seeds can only be induced to germinate by the complete drying and rewetting, or removal of the testa. Other seeds, however, such as the pea, onion, barley and broad bean are not affected in this way and germinate quite readily on removal to a normal atmosphere. In the case of pea and mustard seeds, it has been found that the depressant action of carbon dioxide on germination varies approximately as the square root of the concentration of the gas. Increase of the carbon dioxide of the atmosphere to 20 per cent will inhibit the sprouting of potato tubers but higher concentrations than this bring about permanent injury and eventually death.

Water.—Since water normally composes over 75 per cent of living matter, it is to be expected that water has an important influence on the rate of respiration. This has been found to be the case. The influence of moisture on respiration is shown to the greatest extent in germinating seeds. Thus Bailey* found that when the moisture of sound corn was increased by 15 to 17 per cent, the rate of respiration was increased by as much as 400 per cent. Jacquot and Meyer† found that the seeds of pea-nut, bean and maize had to absorb a certain amount of moisture before carbon dioxide is evolved.

Although an increase in moisture content brings about an increase in the rate of respiration of seeds, under certain conditions a decrease in water-content will bring about an increase in respiration. It was found by A. M. Smith‡ that when the water-content of the leaves of Tropaeolum, Asparagus and snowdrop was decreased to one-half or one-third of the normal value there was increase in the rate of respiration. Similar results were obtained by Maige and Nicolas,§ who found that an increase in the

* *Univ. Minn. Agric. Exp. Stat. Tech. Bull.*, 1921, **3**, 1.
† *Comp. Rend.*, 1925, **181**, 931.
‡ *Brit. Assoc. Rept.*, 1915, p. 725.
§ *Rev. Gen. Bot.*, 1910, **22**, 409.

turgescence of tissues brought about an increase in the rate of respiration, but if the water-content were lowered, there was also an increase in respiration, although to a lesser extent, and with further water loss there was a fall in the respiratory rate.

Acidity.—In certain instances it has been shown that the degree of acidity or alkalinity of the medium can influence the rate of respiration. The majority of these investigations have been carried out with fungi. In *Penicillium crysogenum* it was found by Gustafson* that variations in the pH of the medium from 4 to 8 produced little effect on the normal rate of respiration of this form, i.e. rate of respiration at neutrality (pH = 7). When, however, the pH was increased to 8·8 the rate of respiration fell by over 60 per cent, and remained stationary at this new low value for the remainder of the experimental period. When the pH was reduced to 2·65, a slow rise in the rate of respiration occurred and this then fell away to the normal rate at pH 7. When the pH was reduced to 1·10–1·95 there was a sharp rise of 20 per cent in the respiration rate and this was followed by a fall to below normal. This decrease in rate of respiration proved to be irreversible, whereas the decrease brought about at pH 8·8 was only temporary in nature, and the normal rate was recovered when the pH of the medium was altered to 7.

Salts.—The addition of various salts has been found to affect the rate of respiration. The effect upon respiration of salts depends upon their chemical nature and concentration, and is further complicated by the problem of antagonism.

The addition of phosphate has been found markedly to accelerate the rate of respiration of *Elodea canadensis*. According to Lyon† both the aerobic and anaerobic phase of respiration are influenced by phosphate and similar results were obtained with wheat seedlings.

The additions of the salts of the heavy metals—mercury, copper and silver—depresses the respiratory rate of *Aspergillus niger*, and the toxic action of these salts has been shown to be a constant power of their concentration. On the other hand, the addition of varying concentrations of magnesium chloride, up to 0·01 M, has little effect upon the rate of respiration of *Bacillus subtilis*, similarly, the salts of potassium, sodium and calcium in low concentration did not affect the rate of respiration. Higher concentrations brought about a decrease in the rate, and when

* *J. Gen. Physiol.*, 1920, **2**, 617.

† Ibid., 1924, **6**, 299.

mixtures of these salts were used, antagonism was found and the respiratory rate was not affected.

Temperature.—With increase in temperature there is an increase in the rate of respiration until death supervenes. If respiration were a purely chemical process, the van't Hoff law should apply throughout the range of temperatures employed. The van't Hoff law, however, is only followed over the lower ranges of temperature, for with the incidence of higher temperatures, marked fluctuations occur in the respiratory rate owing to the breakdown of the metabolic machine

Matthaei* obtained the following values for the respiration of 2 gm. of cherry laurel leaves, the respiration rate being measured by the evolution of carbon dioxide per hour at temperatures between 5·8° C. and 33·1° C.

°C.	CO_2	°C.	CO_2
5·8	0·0001	18·2	0·00045
14·2	0·00025	25·7	0·0006
14·3	0·00030	29·2	0·00085
18·1	0·00040	33·1	0·00130

It will be seen from the values enumerated above that there is a gradual rise in the rate of respiration with increase of temperature. At still higher temperatures, marked fluctuations were obtained, and leaves exposed to the same external factors gave divergent results owing to the intervention of some internal factor. The results obtained on isolated cherry laurel leaves, exposed to light and thus able to assimilate, are given below. The respiration rate was again calculated as the amount of carbon dioxide given off per hour by 2 gm. of leaf material:

°C.	CO_2	°C.	CO_2
38·3	0·00205	42·9	0·0015
38·3	0·00230	42·9	0·0014

F. F. Blackman† has found that with cherry laurel leaves the relationship between temperature and respiration is similar to the relationship between photosynthesis and temperature. There is an increase over the initial value with rise of temperature, but this increase is not maintained and falls away with time. The higher the temperature the more rapid the fall. Blackman's "time factor" is of importance in this connection, for it is the duration of time in which the high temperature is allowed to operate that brings about the effect on respiration.

* *Phil. Trans. Roy. Soc.* (*Lond.*), 1904, **197B**, 47. † *Ann. Bot.*, 1905, **19**, 281.

Kuijper* has investigated the relationship between temperature and respiration in the seedlings of lupin, wheat and pea. The seeds were germinated at 20° C. and then exposed to various temperatures. The carbon dioxide evolved was measured per hour over six hour periods. At temperatures up to 10° C. the output of carbon dioxide was constant; from 10° C. to 20° C. there was a gradual rise in the carbon dioxide evolved in seedlings

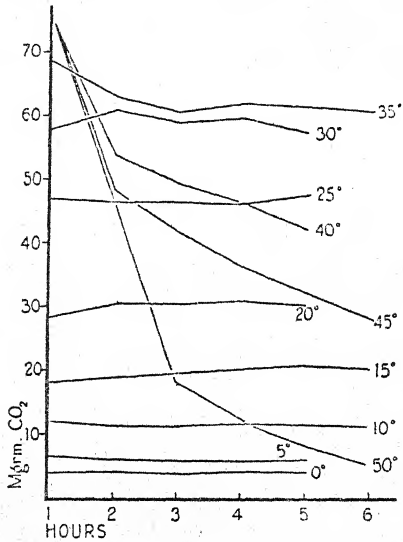


FIG. 34.—The respiration of the pea at different temperatures. (After Kuijper. From Haas & Hill, *Chemistry of Plant Products*, Vol. II.)

of whatever age, and this rise was followed by a gradual fall. From 20° C. to 40° C. fluctuations were found to occur and at still higher temperatures there was a continuous fall (Fig. 34).

Dormant structures show a very considerable difference in respiration rate to that of plants in active development. Müller-Thurgau and Schneider-Orelli† found that the effect of temperature on the respiratory activity of potatoes was not immediate, but that the increase in respiration was only shown 24 hours after their removal to room temperatures. Tubers exposed to a temperature of 40° C. for some hours showed a gradual increase in their respiration after their removal to room temperature and reached their maximum intensity after an interval of

* *Extr. Trav. Bot. Neerland*, 1910, **7**, 130.

† *Flora*, 1910, **101**, 309.

24 hours. Exposures to still higher temperatures (44° C.) brought about a permanent increase in the respiratory rate. It has been suggested that the effect of high temperature in bringing about a permanent increase in the rate of respiration of potato tubers is similar to the effect of old age on respiration, affecting the activity of the enzymes and leaving less sugar for the leucoplasts to fix.

It has already been seen that lowering the temperature lowers the rate of respiration. The effect of low temperatures on the respiration of potato tubers has been investigated by Barker,* who found that apart from the normal lowering of the respiration rate by low temperatures, there is another phenomenon that complicates the problem, which has been termed by him "low temperature depression." This result is apparently brought about by the gradual accumulation at low temperatures of some inhibitory body. The lower the temperature the greater the accumulation of this substance. Thus it is very large at −1° C., moderate at 1° C., and practically nil at 8° C. A second condition, however, was needed for the actual depression of the respiration rate by this inhibitor. This second reaction is brought about by a rise of temperature; it is small at −1° C., great at +1° C. and very rapid at +10° C. Thus the depression in the rate of respiration of potato tubers is brought about by first of all "accumulation by cold" and secondly by "development by heat."

Light.—Besides its indirect effect on the photosynthetic mechanism, light has a direct influence upon respiration. It was found by Spoehr† that the respiration of wheat plants shows a small but definite variation in light and darkness. In normal air the ratio of day to night rate was found to be 1·042, whereas in deionized air the ratio was 1·010.

The effect of ionized air on the rate of respiration of barley has been investigated by Middleton,‡ and by Whimster§ on *Pelargonium zonale*. In both cases the source of ionization was the radio-active element polonium. It was found that barley and *P. zonale* showed an increased rate of respiration in the presence of ionized air. On the other hand, the presence of ionized air apparently does not affect the rate of respiration of fungi. It was found by de Boer‖ that the rate of respiration of

* *Proc. Roy. Soc.* (*Lond.*), 1933, **112B**, 316, 336.
† *Bot. Gaz.*, 1915, **59**, 366.
‡ *Ann. Bot.*, 1927, **41**, 345.
§ Ibid., 1927, **41**, 357.
‖ Ibid., 1930, **44**, 989.

Phycomyces Blakesleeanus and *Polyporus destructor* was not increased in the ionized air.

ANAESTHETICS

A number of investigations have been made from time to time on the effect of various anaesthetics on the respiration rate of plants. Irving,* and also Thoday,† showed that the action of chloroform in small doses of 1 c.c. in 970 c.c. of air increased the rate of respiration, and this increase was followed by a decrease to the normal rate. With stronger doses the increase fell away more rapidly and with still higher doses (10 c.c. in 970) there was no initial increase and the rate of respiration quickly fell to zero. Similar results have been obtained by other investigators.

Gustafson,‡ who used ether, acetone and formaldehyde, found that *Aspergillus niger* showed an increase in respiration and this was followed by a decrease. M. M. Brooks§ showed that ether produced the same result on the respiration of *Bacillus subtilis*.

It remained for E. P. Smith|| to show that the increase in respiration is not immediate in the presence of anaesthetics. Using a "pure line" of wheat, and the pH method of determining the rate of respiration with the indicator phenol red, Smith ascertained that there was an initial decrease, followed by an increase and then a final fall in the respiration of the seedlings. It has been suggested by Smith that the action of the anaesthetic is to decrease the permeability of the plasma-membrane of the cell to carbon dioxide. In the early stages of the process the carbon dioxide is held within the cell, and it is only later that it makes its way out, so that the subsequent increase in respiration may be either partly or wholly illusory.

STIMULATION

The respiration of plants is markedly affected by stimulation such as wounding. Wounding of the potato tuber, for example, brings about a rise in temperature which can be readily demonstrated. H. M. Richards, who worked with potato tubers, carrots, onion, kohl-rabi, radish, cucumber and the leaves of Diervilla and Liriodendron, found in all cases that there was a marked rise in temperature when these tissues were wounded. This so-called "fever-reaction" at its maximum was between two and three

* *Ann. Bot.*, 1911, **25**, 1077. † Ibid., 1913, **27**, 697.
‡ *J. Gen. Physiol.*, 1918, **1**, 181.
§ Ibid., 1918, **1**, 193. || Ibid., 1921, **4**, 157; *Ann. Bot.*, 1924, **38**, 261.

times the ordinary temperature of the air and ran quite a definite course, attaining its maximum some 24 hours after injury and then gradually falling away. In plants with massive tissues, such as the potato tuber, the effect of wounding was local, but in such a structure as the onion, which is composed of leaves, a much wider sphere of action was discovered. It was also found that there was an initial outburst of carbon dioxide production on wounding which rose to a maximum and then fell away. The amount of oxygen consumed was much greater than that required theoretically for the amount of carbon dioxide evolved.

E. F. Hopkins* has put forward the explanation that the increase in respiration shown by a wounded potato is due to increase in sugar-content. He found that the sugar-content of the tubers rose after wounding to a maximum and then fell away.

According to J. White,† pollination also brings about an increase in the respiratory rate. In such plants as Pelargonium, the rise may be as much as 8 times the normal value. Geotropism also influences the rate of respiration. Schley‡ has shown that a geotropically stimulated root respires at a greater rate than an unstimulated one.

ANAEROBIC RESPIRATION

Plants are able to respire for a time in the absence of free oxygen. In these circumstances they are said to respire *anaerobically*. The term *intramolecular respiration* has also been used for this purpose, but since all forms of respiration are essentially intramolecular, it is not a suitable one.

Ethyl alcohol and carbon dioxide are the main products of anaerobic respiration, and the equation for the reaction may be written:

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$$

This equation merely represents the initial and end products of the reaction, and the various stages of the process will be considered below. Anaerobic respiration is a wasteful method of obtaining energy, for the ethyl alcohol produced is a waste product, incapable of further oxidation under the conditions of the reaction.

Many micro-organisms, such as the yeasts, normally respire anaerobically, and the various fermentation processes, as they are

* *Bot. Gaz.*, 1927, **84**, 75.
† *Ann. Bot.*, 1907, **21**, 487.
‡ *Bot. Gaz.*, 1920, **70**, 69.

called, are of great economic importance. It is generally agreed at the present time that there is a relationship between aerobic and anaerobic respiration, and present theories on this subject assume that anaerobic respiration is a preliminary step of respiration, and that normal green plants use the oxygen of the air to complete the oxidation of the products of anaerobic respiration. The possible mechanisms involved in aerobic respiration will be discussed in a separate section when the problem of alcoholic fermentation and other fermentative processes has been considered.

Certain of the higher fungi, such as Agaricus, do not form ethyl alcohol in the absence of free oxygen, although carbon dioxide is produced in considerable amounts. Kostytchev found that the addition of mannite to the pressed juice of Agaricus quickly disappears, but no carbon dioxide is evolved. It has been suggested that the production of carbon dioxide in the anaerobic respiration of Agaricus is through the intermediate formation of some product of oxidation which splits off carbon dioxide by hydrolysis.

Alcoholic Fermentation.—The yeasts (Saccaromyces) are the most important producers of alcoholic fermentation. The main products are ethyl alcohol and carbon dioxide, according to the equation given above, but other substances are also formed in minute amount, such as succinic acid, glycerol, pyruvic acid, acetaldehyde, and the two higher alcohols, isobutyl carbinol and secondary butyl carbinol. These last two substances have been collectively designated *fusel* oil. A high proportion of fusel oil is usually found in cheap spirits, such as that prepared from potatoes.

Different strains of yeasts are not equally efficient as producers of fermentation, and not all sugars are fermented by yeasts. Of the hexoses, *d*-glucose, *d*-fructose, *d*-mannose and *d*-galactose undergo fermentation, but the most suitable of all these substances is *d*-glucose and *d*-fructose, while *d*-galactose is the least readily attacked. The disaccharides, sucrose and maltose must undergo a preliminary hydrolysis to hexoses before fermentation, and this hydrolysis is brought about by the enzymes invertase and maltase which are present in yeast cells.

The properties of alcoholic drinks have been known from very ancient times. Noah after the flood planted a vineyard and prepared wine (Gen. 9. 20), and although various kinds of

alcoholic liquors have been prepared and drunk by the human race in all stages of civilization, it was not until 100 years ago that it was realized that fermentation is a biological process brought about by the activity of micro-organisms.

Liebig explained alcoholic fermentation as being due to the spontaneous splitting of protein in the surrounding solution. Some kind of molecular vibrations were supposed to be set up by the protein molecule in this process which split the sugar molecules into alcohol and carbon dioxide. He considered that only dead yeast cells could bring about fermentation because there was diffusion of protein which caused molecular vibrations by their cleavage and thus brought about the decomposition of sugar. It was supposed that living cells could not bring about fermentation because there was no diffusion of protein from them.

It remained for Pasteur to illustrate the true nature of fermentation. In a series of masterly investigations he showed that yeast is a specific organism of fermentation, and that alcoholic fermentation is impossible in the absence of yeast cells, and that yeast is able to multiply in the absence of oxygen and ferment sugars. Actually, it is only young actively developing yeast cells that are able to grow and ferment in the absence of oxygen, resting yeast cells are unable to do so.

Yeast will not multiply to any great extent in pure solutions of sugar, and nitrogen and mineral substances must be added to the cultures as in the case of other plants. The addition of mineral salts and nitrogen is unnecessary in the fermentation of beer-wort and the juice of grapes by yeast, as these substances are present in these natural products in sufficient amount.

Under certain conditions yeast is able to develop in a medium without the addition of nutrient substances. In these circumstances, alcohol and carbon dioxide are still produced, but the yeast shows a diminution of dry-weight, and eventually dies. In this auto-fermentation of yeast, carbon dioxide and alcohol are formed from the yeast itself. The glycogen stored in the cells is fermented, and at the same time the protein is broken down into crystalloid nitrogen.

The Chemistry of Alcoholic Fermentation.—Alcoholic fermentation by yeast is an enzymic process and several enzymes are involved. It was shown by Buchner that when yeast cells are broken up in water with quartz sand and kieselguhr and then subjected to high pressure, a liquid can be expressed out that is free from

yeast cells and is able to produce active alcoholic fermentation. This solution contains an enzyme known as *zymase*.

It was shown by Harden and Young* that zymase is made up of an enzyme and co-enzyme and that both must be present to bring about fermentation.

The addition of an alkaline solution of phosphate to fermenting liquors produces certain remarkable results. The rate of fermentation is greatly increased, sometimes as much as twenty times the original value. The rate, however, later falls once more to the original value, but it can be increased by the addition of a second dose of phosphate. Harden and Young found that if the solution be boiled when the rate has fallen to the original value after addition of phosphate, no phosphate is precipitated on the addition of uranium acetate, and that the phosphate is in organic union with hexose as a hexosediphosphate.

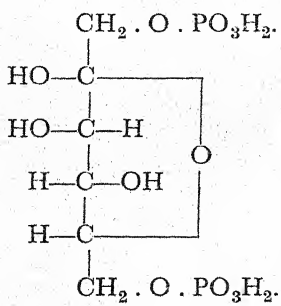
Harden and Young concluded that alcoholic fermentation takes place in two stages. In the first stage a hexosediphosphate is formed:

$$\text{(i)}\quad 2C_6H_{12}O_6 + 2R_2HPO_4 = 2CO_2 + 2C_2H_5OH + C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O$$

The second reaction consists of the hydrolysis by water of the hexosediphosphate so formed into phosphate and hexose:

$$\text{(ii)}\quad C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = 2R_2HPO_4 + C_6H_{12}O_6$$

It has been shown by Robison and Morgan† that whatever may be the nature of the hexose used for fermentation, the same hexosediphosphate is formed and is a fructosediphosphate and the fructose in the molecule of the ester is probably in the active or γ-form:



γ-fructosediphosphate

* *Proc. Roy. Soc.* (*Lond.*), 1906, **77B**, 405; **78B**, 369; 1908, **80B**, 299; *Centralbl. Bakt.*, 1910, **26**, 178.

† *Biochem. J.*, 1928, **22**, 1277; 1930, **24**, 119.

The problem of the hexosephosphatic esters in alcoholic fermentation is a complex one. It had previously been shown by Robison* that not only was a hexosediphosphatic ester present but that a monophosphatic ester was also formed, and the function of these hexosephosphatic esters in alcoholic fermentation will be more clearly understood after the scheme of Neuberg for the formation of carbon dioxide and alcohol from hexose by yeast is discussed.

The chemical scheme for alcoholic fermentation suggested in 1913 by Neuberg and Kerb† postulated the intermediate formation of methylglyoxal. The oxidation and reduction of this substance was assumed to be brought about by a series of Canizzaro reactions of the various aldehydes produced as intermediate products.

The first step in the reaction was the splitting of the hexose into two molecules of methylglyoxal (pyruvic aldehyde):

$$C_6H_{12}O_6 - 2H_2O = \underset{\substack{\text{Methylglyoxal}\\ \text{aldol}}}{C_6H_8O_4} = \underset{\text{Methylglyoxal}}{2CH_3 . CO . CH : O}$$

$$\text{or} \quad 2CH_2 : C(OH) . CH : O$$

Under the influence of water, two molecules of methylglyoxal underwent simultaneous oxidation and reduction, the products of the reaction being pyruvic acid and glycerol:

$$\begin{array}{lll} CH_2 : C(OH) . CH : O + H_2 + H_2O & = & \underset{\text{Glycerol}}{CH_2OH . CHOH . CH_2OH} \\ \qquad\qquad\qquad\qquad \| & & \\ CH_3 . CO . CH : O \qquad\quad O & = & \underset{\text{Pyruvic acid}}{CH_3 . CO . COOH} \end{array}$$

The pyruvic acid formed in this reaction was considered to be converted into acetaldehyde and carbon dioxide by the enzyme carboxylase:

$$CH_3 . CO . COOH = CH_3 . CH : O + CO_2$$

The acetaldehyde formed in this manner was considered to undergo a second Canizzaro reaction under the influence of water to ethyl alcohol and simultaneously a molecule of methylglyoxal was oxidized to pyruvic acid:

$$\begin{array}{lll} CH_3 . CH : O & H_2 & = CH_3 . CH_2 . OH \\ & + \| & \\ CH_3 . CO . CH : O & O & = CH_3 . CO . COOH \end{array}$$

The pyruvic acid formed in this way is then converted into more acetaldehyde and carbon dioxide.

* *Biochem. J.*, 1922, **16**, 809.

† *Biochem. Zeit.*, 1913, **58**, 158.

The connection of the hexosephosphate esters formed in the initial stages of alcoholic fermentation and Neuberg's scheme of fermentation must now be considered. It has been known for a long time that there is an initial lag period between the addition of phosphate and the evolution of carbon dioxide, and that this lag period can be reduced by the addition of various hydrogen acceptors, such as methylene blue. Since the Neuberg scheme postulates that the hexose molecule is converted into two molecules of methylglyoxal as the preliminary stage in the reaction, it was suggested by Boyland* that the sequence of reactions may be as follows:

$$(1)\quad 3C_6H_{12}O_6 + 2R_2HPO_4 = 2C_6H_{11}O_5(PO_4R_2) + 2CH_3 . CO . CH \cdot O + 4H_2O$$

$$(2)\quad 2C_6H_{11}O_5(PO_4R_2) + C_6H_{12}O_6 + 2R_2HPO_4 = 2C_6H_{10}O_4(PO_4R_2)_2 + 2CH_3 . CO . CH : O + 4H_2O$$

$$(3)\quad CH_3 . CO . CH : O + CH_3 . CH = O + H_2O = CH_3 . CO . COOH + C_2H_5OH$$

$$(4)\quad CH_3 . CO . COOH = CH_3 . CH : O + CO_2$$

Equation (3) is the only one that is likely to be affected by the presence of hydrogen acceptors, and since the presence of hydrogen acceptors has a marked effect upon the evolution of carbon dioxide, this reaction is probably the slowest or controlling stage in the beginning of the reaction with phosphate. The recent investigations of Embden,† Meyerhof and others, however, have shown that the postulation of the formation of methylglyoxal as an intermediate is not necessary in alcoholic fermentation, and it will be necessary to discuss these fresh views of the chemical mechanism of yeast fermentation.

The Embden-Meyerhof scheme of alcoholic fermentation centres round the formation of pyruvic acid. It will be remembered that Robison isolated a hexosemonophosphate ester from yeast juice, and Embden isolated a similar ester from muscle. Later, Meyerhof and Lohmann were able to show that this ester is not a uniform substance, but a mixture consisting approximately of three parts of an aldose monophosphoric ester and one part ketose monophosphoric ester.

It was next established by Lohmann‡ that when muscle

* *Biochem. J.*, 1930, **24**, 703.
† *Klin. Woch.*, 1933, **12**, 213.
‡ *Biochem. Zeit.*, 1930, **222**, 324.

tissue is minced up in the presence of sodium fluoride, and glycogen or starch added, a part only of the hexosephosphoric ester which accumulates is the true Harden-Young fructose-1 : 6-diphosphoric acid. Sometimes even the whole and always a considerable part was found to be present in the form of an ester of the same elementary composition, but showing a marked resistance to hydrolysis by acids. So pronounced was this resistance to acid hydrolysis that Lohmann applied the term, "unhydrolysable" ester to this fraction. It was found that the monophosphoric esters isolated by Robison and Embden could be converted into this resistant ester by taking up one equivalent of phosphate from muscle extracts containing fluoride.

It was discovered by Embden* that one constituent of Lohmann's "unhydrolysable" ester is phosphoglyceric acid,

$$(HO)_2 . PO . O . CH_2 . CH(OH) . COOH,$$

and later Meyerhof† found that the other constituent was *l*-α-glycerophosphoric acid,

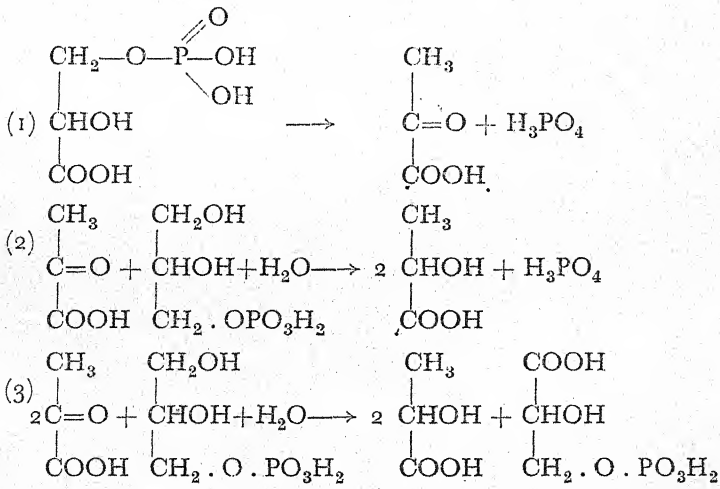
$$(HO)_2 . PO . O . CH_2 . CH(OH) . CH_2 . OH.$$

The formation of pyruvic acid under anaerobic conditions in minced muscle has been described by several investigators. It was shown by Case,‡ for example, that when starch and sodium sulphite are added to an aqueous extract of muscle there is an increase in the production of pyruvic acid, whereas, if lactate and sulphite be added, only a small amount of pyruvic acid is formed. It is therefore clear that the pyruvic acid must be formed from carbohydrate and the quantity of the acid is increased if it be "fixed" with sulphite. It had been found by Embden that if phosphoglyceric acid and glycerophosphoric acid be simultaneously added to muscle tissue there is a considerable amount of lactic acid formed. Furthermore, when pyruvic acid and α-glycerophosphoric acid were added together to muscle, lactic acid was formed, the amount of lactic acid formed being equivalent to twice the pyruvic acid which had disappeared. From these results Embden formulated a scheme for the formation of lactic acid in muscle. He did not consider that pyruvic acid and glycerophosphoric acid react directly to give two molecules of lactic acid, but instead form lactic acid and a triosephosphoric acid; the latter then undergoes rearrangement to give phospho-

* *Biochem. Zeit.*, 1933, **222**, 389.
† *Nature*, 1933, **132**, 337, 373.
‡ *Biochem. J.*, 1932, **26**, 759.

glyceric acid and glycerophosphoric acid. The resulting phosphoglyceric acid then gives rise to pyruvic acid which reacts once more with glycerophosphoric acid until the whole has been converted into lactic acid.

Further evidence of the course of breakdown of carbohydrate by muscle was obtained by a study of the behaviour of triosephosphoric acid. H. O. L. Fischer and Baer* were able to synthesize glyceraldehyde-γ-phosphoric acid and the dextro-component of their racemic compound has been shown to undergo fermentation by yeast with a velocity equal to that of glucose of the same molar concentration. Embden found that if this compound were added to muscle it was converted into lactic acid, and Meyerhof and Kiessling† were able to show that in muscle extract this substance is converted into phosphoglyceric acid and glycerophosphoric acid, and in the presence of sulphite, pyruvic and glycerophosphoric acids are formed. On the Embden scheme for the formation of lactic acid in muscle, fructose-diphosphoric acid is first converted into triosephosphoric acids, glyceraldehyde phosphoric acid and dihydroxyacetone phosphoric acid. From these are obtained phosphoglyceric acid and glycerophosphoric acid and the phosphoglyceric acid then gives rise to pyruvic acid and phosphoric acid. The pyruvic acid so formed reacts with glycerophosphoric acid to give lactic acid and triosephosphoric acid, thus:



* *Ber. deut. chem. Ges.*, 1932, **65B**, 337.
† *Biochem. Zeit.*, 1933, **264**, 46; *Naturwiss.*, 1933, **21**, 223.

The question now arises as to how this scheme may be applied to alcoholic fermentation by yeast. It was shown by Neuberg and Kobel* that yeast and the lactic acid bacterium (*Bacillus Delbrücki*) are able to form pyruvic acid from glycerophosphoric acid, and yeast also gives rise to some acetaldehyde, while Nilsson† found that when dried yeast was allowed to act upon hexosediphosphoric acid in the presence of sodium fluoride, glucose and acetaldehyde phosphoglyceric acid is formed. It was later demonstrated by Meyerhof and Kiessling that with yeast maceration juice containing sodium fluoride, hexosediphosphoric acid is converted as in muscle extract into Lohmann's "unhydrolysable" ester, that is to say, into an equimolecular mixture of α-glycerophosphoric acid and phosphoglyceric acid, and that glyceraldehyde-γ-phosphoric acid behaves in the same way. In fresh yeast extract, phosphoglyceric acid is converted to acetaldehyde, carbon dioxide and phosphoric acid. The acetaldehyde is reduced to ethyl alcohol by the triosephosphoric acid arising from the interaction of glucose and hexosediphosphoric acid, and phosphoglyceric acid is simultaneously formed:

$$(HO)_2 . PO . O . CH_2 . CH(OH) . CH : O + CH_3 . CH : O + H_2O$$
$$\rightarrow (HO)_2 . PO . O . CH_2 . CH(OH) . COOH + CH_3 . CH_2 . OH$$

According to Meyerhof, the main difference between lactic acid formation in muscle and alcoholic fermentation by yeast lies essentially in the difference in the fate of the pyruvic acid. In muscle extract it reacts with glycerophosphoric acid and is reduced to lactic acid, whereas in yeast it is split by carboxylase into carbon dioxide and acetaldehyde, and the latter is then reduced to ethyl alcohol. The Embden-Meyerhof scheme of yeast fermentation is summarized below:

1 Hexosediphosphoric acid + 1 Glucose + 2 Phosphoric acid

↓

4 Triosephosphoric acid

↓ ↓

2 Glycerophosphoric acid + 2 Phosphoglyceric acid

↓

2 Pyruvic acid + 2 Phosphoric acid

* *Biochem. Zeit.*, 1933, **260**, 241.
† *Arkiv. Kemi. Min. Geol.*, 1930, **10**A, No. 7.

1 Glucose + 2 Acetaldehyde + $2CO_2$ + 2 Phosphoric acid

↓

2 Triosephosphoric acid + 2 Acetaldehyde

↓

2 Ethyl alcohol + 2 Phosphoglyceric acid

Butyric Acid Fermentation.—The organisms concerned in butyric acid fermentation are anaerobes, although it has been stated that certain aerobic bacteria produce butyric acid. Butyric acid fermentation was first discovered by Pasteur, and he was able to show that the organisms concerned are obligate anaerobes.

A number of bacteria are capable of butyric acid fermentation. The genus Clostridium is one such form. *Clostridium butyricum* was described by Prazmowski as the form investigated by Pasteur. *C. Pasteurianum*, it will be remembered, is able to fix molecular nitrogen (see Chapter X) and also brings about butyric acid fermentation. Yet another organism that is able to bring about butyric acid fermentation is the non-motile bacillus *B. butyricus*, which is a strongly anaerobic form.

Theoretically, in butyric acid fermentation, for every molecule of sugar decomposed, two molecules of carbon dioxide and two molecules of hydrogen are evolved and one molecule of butyric acid formed:

$$C_6H_{12}O_6 = CH_3 . CH_2 . CH_2 . COOH + 2CO_2 + 2H_2$$

A number of by-products have been described in butyric acid fermentation, principally ethyl alcohol, acetic acid, and lactic acid. The butyric acid bacteria are catholic in their taste of substrate, and will ferment not only sugars, but also starch, lactic acid, and glycerol. Bacteria have now been isolated that are able to decompose cellulose to butyric acid and acetic acid and carbon dioxide and hydrogen or methane. Which of these two latter substances is produced depends upon cultural conditions. If the initial inoculation be first warmed to 75° C., hydrogen is principally formed.

Butyric acid fermentation is common in nature, and occurs in mud free from oxygen and in moorland soils.

Lactic Acid Fermentation.—In lactic acid fermentation one molecule of sugar is broken up into two molecules of lactic acid. Milk is the best substrate for demonstrating lactic acid fermentation.

The first step in the process is the hydrolysis of milk sugar, lactose, to one molecule of glucose and one molecule of galactose. The bacteria concerned then break down these hexoses into lactic acid.

Two groups of lactic acid fermenting organisms have been distinguished. The first group, of which *Bacterium lactis acidi* is a typical example, are anaerobes and bring about lactic acid formation in the deeper layers of milk according to the equation:

$$C_6H_{12}O_6 = 2CH_3 . CH(OH) . COOH$$

About 98 per cent of the total amount of lactic acid formed in milk is brought about by this type of lactic acid fermentation.

The second group of lactic acid-forming organisms to be found in milk are aerobes, of which *Bacterium acidi lactici* is an example. This form develops in the upper layers of the milk, and produces not only lactic acid, but also ethyl alcohol and acetic acid. *Bacterium lactis aerogenes* not only forms lactic acid, but also ethyl alcohol, acetic acid and carbon dioxide and hydrogen and methane. The chemistry of lactic acid formation is not understood, and the facts are confusing and contradictory. Thus some forms produce *d*-lactic acid, others *l*-lactic acid and some *dl*-lactic acid. Again *B. lactis acidi* gives rise to *dl*-lactic acid when it is grown in cow's milk, but in artificial media it forms *l*-lactic acid.

The accumulation of the products of lactic acid fermentation has a powerful effect upon the rate of the reaction. *B. acidi lactici*, for example, is unable to tolerate a higher concentration of lactic acid than 0·58–0·8 per cent in the medium, while *B. lactis acidi* is able to tolerate a slightly higher range of concentration of the acid (0·54–1·25 per cent). In the presence of calcium carbonate, however, the sugar is entirely broken down.

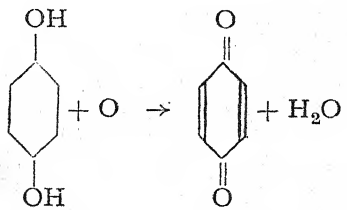
THE OXIDATION MECHANISM OF THE CELL

We have already seen that it is through respiration that the plant or other living organism is able to obtain its necessary supplies of energy, so that it is able to carry out its metabolic activities. In its narrow sense, respiration is the absorption of oxygen and the evolution of carbon dioxide. But the term respiration has a wider meaning if it be used to cover all the oxidative processes of living organisms in which energy is released, and will be employed in this sense here.

It is obvious that in the aerobic respiration of carbohydrate to carbon dioxide and water the process must take place in a series of steps, for the physiological combustion of hexose involves the decomposition of a substance with six carbon atoms to carbon dioxide, a substance with only one atom of carbon in the molecule. Furthermore, glucose or other hexose is not oxidized by atmospheric oxygen at ordinary temperatures at which living organisms are able to exist, and it will be necessary to examine the various mechanisms in the cell which are able to bring about this oxidation at the restricted range of temperature tolerated by living organisms.

From the chemical standpoint oxidation is a complex process, and involves more than the mere addition of oxygen to a substance. Oxidation involves the addition of oxygen or any electro-negative element to a substance, or the subtraction of hydrogen or any electro-positive element from a substance.

The usual example given of this latter type of oxidation involving the subtraction of hydrogen from a substance is the formation of quinones from dihydroxy phenols. Thus the formation of quinone from quinol:



is as much a process of oxidation as the burning of magnesium in air or oxygen to form its oxide.

In living cells this latter type of oxidation is of common occurrence, and the presence of some substance which is able to take up the hydrogen is necessary, and the term *hydrogen acceptor* has been given to this substance.

In this discussion of the various oxidation mechanisms of the living cell, the physical structure as well as the chemical viewpoint must be considered. Warburg* attaches much importance to structure in cellular oxidations. On his view successful oxidation can only take place if suitable surfaces are offered for the activity of the surface catalysts taking part in oxidation. A few

* *Biochem. Zeit.*, 1921, **119**, 134; *Zeit. Electrochem.*, 1922, **28**, 70.

of Warburg's experimental results may be considered to demonstrate the reason for his emphasizing the physical aspect of the problem. For instance, he showed that when cystine and certain other amino-acids were shaken at ordinary temperatures and in the presence of air with blood charcoal, they were oxidized to ammonia, carbon dioxide and water.

The presence of iron was found to be of first importance in these reactions. For example, if charcoal prepared from benzoic acid were used in place of blood charcoal, scarcely any oxidation took place, whereas blood charcoal which contains iron was very active.

Various narcotics, such as phenyl urethane and dimethyl urea, were found to act upon this so-called "charcoal model" in the same manner as on the living cell, inasmuch as they depressed the rate of oxidation. Warburg considers the action of narcotics upon the living cell to be due to their adsorption upon the oxidizing surfaces so that the oxidative capacity of the latter is reduced, and he was able to show that dimethyl urea, for example, is readily adsorbed upon charcoal.

Warburg has visualized the cell as being composed of a mosaic of areas consisting of iron-bearing and non-iron-bearing surfaces, and suggests that it is upon the iron-bearing surfaces that cellular oxidations take place. Further evidence for his view of the importance of iron in cellular oxidations is given by the fact that when pure charcoal is impregnated with an iron salt and then heated to red heat, the oxidative capacity of this charcoal is greatly increased, and narcotics act upon it in the same way as blood charcoal. It has been known for a long time that potassium cyanide in minute concentration can bring all oxidations in the living cell to a standstill without effecting lasting injury. If the cyanide be washed out, the oxidative activity of the cell is recovered. Warburg showed that the respiration of sea-urchin eggs ceases in the absence of iron, and the suggestion has been made that in the experiment described above, the cyanide combines with the iron present in the cell, and brings oxidation to a standstill. Since iron and cyanide combine *in vitro* to give complex salts of the type of the ferro- and ferricyanides, which are stable bodies, it is difficult to understand why the reverse process brought about by the mere washing of the tissues should take place.

The oxidation of amino-acids is readily explained on Warburg's theory, but difficulties arise when the theory is applied to the

oxidation of carbohydrates. Neither glucose nor fructose are oxidized to any marked extent by blood charcoal. On the other hand, the hexosephosphates are oxidized, and it is possible that this may be of some significance in the oxidation of carbohydrates if the present view of aerobic respiration be correct, namely, that anaerobic respiration is one stage of the process (see below).

On the Warburg view, iron acts as oxygen carrier or activator in the cell, and he states that molecular oxygen is unable to act as a hydrogen acceptor unless first activated by iron. According to Wieland,* however, it is the activation of hydrogen that is the first essential condition for cellular oxidation. It will be seen that the Warburg and Wieland views are quite opposed to one another.

There are a large number of oxidation processes known in organic chemistry which depend upon the removal of hydrogen in the presence of a hydrogen acceptor. The oxidation of aldehyde to acid is a case in point. This reaction can take place in the absence of oxygen, so long as a suitable hydrogen acceptor is present as well as a suitable catalyst. In this instance methylene blue is a suitable acceptor and palladium black a suitable catalyst. In the presence of methylene blue, hydrogen is removed from the molecule of aldehyde and the dye is reduced to the leucobase: Water must be present for the reaction to take place, and it is considered that the first part of the reaction consists in the formation of a hydrate which is then oxidized by dehydrogenation:

$$CH_3 . CH : O + H_2O = CH_3 . C\begin{cases} OH \\ OH \\ H \end{cases}$$

$$CH_3 . C\begin{cases} OH \\ OH \\ H \end{cases} + MB = CH_3C\begin{cases} OH \\ \\ O \end{cases} + MBH_2$$

Carbon compounds with two hydroxyl groups attached to the same carbon atom are as a rule unstable, but the exception is chloral hydrate,

$$CCl_3 . CH\begin{cases} OH \\ OH \end{cases}$$

* *Ber. deut. chem. Ges.*, 1912, **45**, 679, 2613; 1913, **46**, 333.

This substance is readily oxidized to the corresponding acid in the presence of methylene blue and palladium black, whereas the aldehyde itself, $CCl_3 . CH : O$, is unaffected. This fact certainly gives strong support to the mechanism of oxidation described above.

Oxidation reactions brought about by enzymes are considered by Wieland to be due to the activation of the hydrogen of the substrate, and the hydrogen is then removed in the presence of some suitable acceptor such as atmospheric oxygen. On his view the enzymes are really activators of hydrogen and he has called them *dehydrases*.

Wieland has shown that the formation of vinegar from ethyl alcohol in the presence of *Bacterium aceti* is a dehydrogenation oxidation. Freshly washed cultures in the presence of alcohol and methylene blue quickly reduce the dye to the leuco-base and in a few days give sufficient acetic acid for identification.

A sprig of Elodea, placed in a tube filled with water from which carbon dioxide has been expelled and which has been coloured with methylene blue and then placed in the dark, will decolourize the dye. If the tube be placed in the light, the reduced methylene blue is oxidized to the dye once more by the oxygen evolved in assimilation. In the dark the methylene blue acts as a hydrogen acceptor during respiration and is reduced to the leuco-base.

The "Schardinger reaction" for distinguishing between boiled and unboiled milk has also been investigated by Wieland. This test is made as follows: Milk is warmed in a test-tube with methylene blue in the presence of a drop of acetaldehyde. If the milk has not been previously boiled the dye is decolourized, whereas if it has been boiled there is no colour change. Wieland supposes that there is a special dehydrase present in the milk which activates the hydrogen of the aldehyde hydrate and the methylene blue then plays the part of hydrogen acceptor.

Thunberg* removed by washing the reducing substance present in frog muscle which reduces methylene blue. He then found that the addition of certain organic compounds, notably succinic acid, brought about the reduction of the dye, while others, such as propionic acid, which is known not to yield its hydrogen readily, failed to bring about any such reduction. On Thunberg's view, reduction of the methylene blue is brought about by the

* *Skand. Archiv. Physiol.*, 1920, **40**, 1; *Naturwiss.*, 1922, **10**, 417.

giving up of hydrogen by some donor and the transport of the hydrogen by an enzyme to the acceptor, in this case methylene blue. Thunberg goes so far as to consider that hydrogen is the primary fuel of the cell and that substances which do not affect methylene blue in the above experiment cannot be intermediate stages in the metabolism of the organism.

The main point of the Wieland theory of oxidation is the activation of hydrogen, so that the substance activated becomes for the time being a hydrogen donator. Wieland's, views have been criticized by Warburg, who has pointed out that the activated hydrogen, when finally oxidized, should give rise to hydrogen peroxide and not water. On the other hand, as Wieland has pointed out in reply to this objection, all aerobic cells contain the enzyme catalase which oxidizes hydrogen peroxide to oxygen and water as it is formed. Wieland explains the action of cyanides as being due to their destructive effect on catalase. In these circumstances hydrogen peroxide is able to accumulate in the cell and exert its full toxic influence. There is, however, one very strong objection to this view. The action of cyanides is temporary, whereas on Wieland's view it should be permanent.

The emphasis that Warburg has placed upon the all-importance of iron in cellular oxidations finds a serious objection in the fact that the purest preparations of the oxidizing enzyme, peroxidase, prepared by Willstätter, contain no iron. There are also a number of other objections. It was found by Moureu and Dufraisse* that hydrocyanic acid is able to act as a powerful depressant of oxidation in the absence of iron. It was further found by these authors that not only hydrocyanic acid, but also ferrocyanides, which, according to Warburg, should have no action, are depressants of oxidation.

Glutathione.—In 1921 Sir F. G. Hopkins isolated† from yeast cells a substance to which he gave the name of glutathione. This substance has been shown to have a wide distribution in the plant world. Chemically, glutathione is a tripeptide of glutamic acid, glycine and cysteine, and has the following constitution:

$$\mathrm{COOH-CH(NH_2)\,.\,CH_2\,.\,CH_2\,.\,CO\,.\,NH\,.\,CH(CH_2\,.\,SH)\,.\,CO\,.\,NH\,.\,CH_2-COOH}$$

γ-glutamyl-cysteinyl-glycine

* *Compt. rend.*, 1922, **174**, 258; 1926, **183**, 685.
† *Biochem. J.*, 1921, **15**, 286; *Lancet*, 1923, **101**, 1251; *J. Biol. Chem.*, 1929, **23**, 982.

Glutathione can either act as a reducing agent yielding hydrogen and itself becoming oxidized, or as an oxidizing agent accepting hydrogen and itself becoming reduced. Thus, in the presence of a hydrogen acceptor, glutathione gives up hydrogen:

$$2G—SH + \text{Acceptor} = G—S—S—G$$

while in the presence of a hydrogen donator the glutathione (oxidized form) plays the part of hydrogen acceptor:

$$G—S—S—G + 2H = 2G—SH$$

Respiratory Pigments.—Palladin considers that certain pigments which he has termed "respiration chromogens" have a wide distribution in the plant world and play an important part in the oxidation process of plant cells. To obtain these chromogens, the plant tissues are boiled with water and the liquid filtered. To the filtrate peroxidase and hydrogen peroxide is added when a red colouration is produced, due to the formation of the respiration pigment from the oxidation of the chromogen. This is converted by further oxidation into a black or violet substance.

The respiration chromogens are said to be present in the form of prochromogens, which are possibly of the nature of glucosides, and these are oxidized by the enzymes present in the cells to the chromogens themselves. The chromogens remove hydrogen formed in respiration by forming water. On Palladin's view all the carbon dioxide of respiration is anaerobic in origin, and during the anaerobic phase of respiration (see below) the chromogens are reduced by substances formed during this phase. The next stage is the oxidation of these reduced products by oxygen. Thus if R represents the respiration chromogen, the following reaction takes place:

$$(1)\ C_6H_{12}O_6 + 6H_2O + 12R = 6CO_2 + 12RH_2$$
$$(2)\ 12RH_2 + 6O_2 = 12R + 12H_2O$$

The anaerobic phase of the reaction is represented in equation (1) and the aerobic phase by equation (2). The main difficulty encountered with this theory, which probably makes its universal acceptance impossible, is the fact that in a large number of the higher plants oxidase systems (see below) are absent.

Nevertheless, three pigments have been discovered in plant tissues which carry out oxidation reactions. P. Haas and Hill*

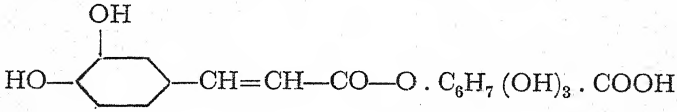
* *Biochem. J.*, 1925, **19**, 233; *Ann. Bot.*, 1925, **39**, 861; 1926, **40**, 709.

have isolated a colourless chromogen from the leaves of *Mercurialis perennis* which takes up oxygen with great avidity. This substance has been called by them *hermidon*. It has been found that hermidon undergoes oxidation in two stages. In the first stage it gives rise to a fugitive blue compound, cyanohermidon, and in the second stage it gives rise to a yellow body, which is stable, crysohermidon. The same volume of oxygen is fixed in the two stages of the reaction.

Cyanohermidon is reduced by such substances as sodium hydrosulphite, and if the solution be shaken with air the colour is once more restored. Crysohermidon is only reduced by fairly powerful reducing agents such as aluminium-mercury couple.

Haas and Hill consider that hermidon is concerned in respiration of *Mercurialis perennis*, since it is most abundantly present at times when the respiration of this plant is at its height in spring and summer. These authors suppose that hermidon is oxidized to cyanohermidon and this in turn is immediately reduced to hermidon once more, losing its oxygen to some reducing agent which may be a metabolite.

At the present time very little is known about the oxidation of fats and proteins in the plant cell. But a depside, chlorogenic acid, has been isolated by Oparin* which may throw some light on this problem. Chlorogenic acid has a wide distribution in the plant kingdom, but has not been found in lichens, which are the chief source of depsides. Chlorogenic acid possesses the following constitution:



It is optically active, is not precipitated by gelatin, and gives a green colour with ferric chloride. It is oxidized by atmospheric oxygen with loss of four atoms of hydrogen and gives rise to a green pigment. This pigment is able to play the part of a hydrogen acceptor. Chlorogenic acid is said to be an active oxidizing agent of α-amino-acids, peptides and peptones:

$$R . CH(NH_2) . COOH + O \rightarrow R . CH : O + CO_2 + NH_3$$

A third pigment with respiratory activities that has been discovered is known as *cytochrome*. This is a thermostable body whose

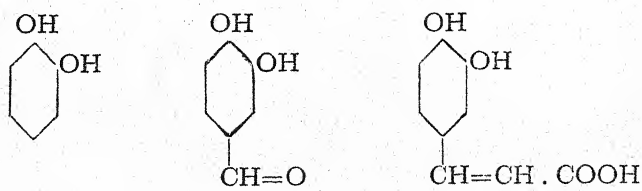
* *Biochem. Zeit.*, 1921, **124**, 90; 1927, **182**, 155.

reactions have been very fully investigated by Keilin.* Cytochrome has a wide distribution in the animal kingdom and has also been shown to be present in plant cells. The reactions of cytochrome and its possible function in the oxidation mechanism of the cell will be considered in the section on oxidizing enzymes.

Oxidation Enzymes of the Cell.—It has long been known that there are a number of enzymes concerned with the oxidation processes of the living cell. The chief of these are oxidase, peroxidase, catalase, zymase, carboxylase and tyrosinase.

Oxidase.—The oxidase system of enzymes is able to bring about oxidation by means of free oxygen. A delicate test that is used to determine whether an oxidase is present in tissues is a 1 per cent alcoholic solution of guaiacum tincture. If an oxidase be present a blue colour is developed.

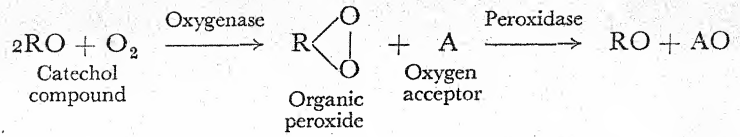
Wheldale Onslow† discovered that in various tissues which give this direct reaction with tincture of guaiacum, catechol compounds are also present. When solutions of catechol derivatives are exposed to air they slowly undergo auto-oxidation to give brown-coloured products. At the same time peroxide, either an organic peroxide or hydrogen peroxide itself, is also said to be formed. In plants which give the direct guaiacum reaction, an enzyme called oxygenase is said to be responsible for the catalysis of the oxidation of catechol derivatives. The most important catechol compounds that have been isolated from plant tissues are catechol itself, protocatechuic acid and caffeic acid:



Catechol Protocatechuic Acid Caffeic Acid

This catechol-oxidase system is said to react in the following way. The catechol compound in the presence of free oxygen and the enzyme oxygenase react to give an organic peroxide. The organic peroxide and some oxygen acceptor and another enzyme, peroxidase (see p. 377), give rise to the catechol compound once more and the oxygen acceptor is oxidized:

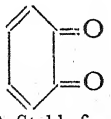
* *Proc. Roy. Soc. (Lond.)*, 1925, **98B**, 312; 1926, **100B**, 129; 1929, **104B**, 206.
† *Biochem. J.*, 1919, **13**, 1; 1920, **14**, 538.



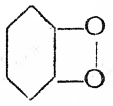
The catechol compound can be extracted with alcohol when the peroxide peroxidase system is prevented and the peroxidase is left in the tissues as a residue. The system, however, can be reformed by the addition of the aromatic compound.

Oxygenase does not appear to be universally present in all plants. Onslow examined some 300 species of flowering plants and only 63 per cent were found to contain oxygenase.

Szent-Györgyi* considers that peroxidases are not specific enzymes and are merely attenuated forms of oxidase, and in his view there is no necessity to postulate the existence of a peroxidase in the direct oxidase system described above. All that is necessary is an oxidase and a substrate containing a catechol derivative. The oxidase catalyses the oxidation of the catechol compound to an *o*-quinone, of which there are two possible types, a stable and an unstable form:

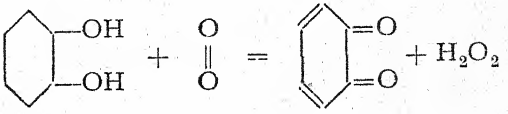


(1) Stable form



(2) Unstable

In the absence of catechol compounds, hydrogen peroxide or a *p*-hydroxy derivative substrate is present and this is oxidized to a *p*-quinone. Onslow and M. E. R. Robinson† have now accepted this view and have suggested that molecular oxygen plays the part of hydrogen acceptor in the reaction and the phenolic derivative is oxidized to a quinone and at the same time hydrogen peroxide is formed:



If this be the case, then oxygenase must act as a dehydrogenase.

Peroxidase.—Certain tissues, which do not give the direct oxidase reaction with tincture of guaiacum, will give a blue colour if hydrogen peroxide is also added. Horse-radish, for

* *Biochem. Zeit.*, 1925, **162**, 399.

† *Biochem. J.*, 1926, **20**, 1138.

example, will not give a blue colour if treated alone with guaiacum tincture, but the addition of hydrogen peroxide brings about the development of the blue colour. The name peroxidase has been given to this enzyme. Peroxidase is only able to carry out its oxidative functions in the presence of a peroxide.

Willstätter* and his co-workers have been able to prepare extremely active preparations of peroxidase from horse-radish roots by means of adsorption of the enzyme on alumina. The early preparations were found to contain nitrogen and iron, and a correlation was found to obtain between the iron-content and activity of the enzyme. Later, however, purer preparations were obtained, which were still found to contain nitrogen, but the iron-content was reduced to the low value of 0·06 per cent, so that enzymic activity is not to be correlated with the amount of iron present.

The function of cytochrome in cellular oxidations can be more properly considered here. It was shown by Keilin that yeast contains four haematin compounds, an unbound protohaematin and three cytochromes, *a′*, *b′* and *c′*. These substances were found to undergo oxidation and reduction independently of one another, and remain in the oxidized state in the presence of oxygen, but when the supply of oxygen is cut off they are reduced.

According to Keilin these haematin compounds are responsible for the peroxidase reactions of bacteria. Aerobes, such as *Bacillus subtilis*, contain a thermostable peroxidase system and cytochrome, whereas anaerobes do not give the peroxidase reaction and contain no cytochrome.

Of the four haematin compounds described by Keilin in yeast, cytochrome *a′* and *c′* are not auto-oxidizable, whereas *b′* and protohaematin are. Keilin considers that cytochrome plays an important part in the respiratory activity of the cell. On his scheme, dehydrases first of all activate the hydrogen of the molecules of the substrate. He nowhere specifies the nature of the substrate which is possibly one or more of the intermediate derivatives of anaerobic respiration. Once these substances have been activated they become hydrogen donors. Cytochrome *a′* and *c′* now come into play as hydrogen acceptors and are reduced. The next stage is the reoxidization of reduced cytochrome *a′* and *c′* by the indophenol oxidase, so that they are able once more to act as hydrogen acceptors.

* *Annalen*, 1918, **416**, 21; 1923, **630**, 269.

This indophenol oxidase is a thermolabile oxidase system discovered in yeast cells by Keilin. It is able rapidly to oxidize *p*-phenylenediamine to a dark purple quininoid compound. It is destroyed at 70° C. and is inhibited by potassium cyanide and hydrogen sulphide.

Activation of the hydrogen of the molecules of the substrate, acceptance of this hydrogen by cytochrome *a'* and *c'*, and oxidation of these reduction products by the indophenol oxidase are considered to be the main respiration mechanism; but respiration can also take place by means of protohaematin and cytochrome *b'*. These bodies are auto-oxidizable, and an oxidase system is not necessary to reconvert them into hydrogen acceptors after they have been reduced. This second system has been termed by Keilin "residual respiration."

There are several objections to the acceptance of Keilin's scheme. In the first place it has not been proved that it plays any part in the oxidation of carbohydrates in the green plant, and the catecholoxidase system, which Keilin regards as being functionally the same as the indophenol-oxidase system of yeast and animal cells, only occurs in 63 per cent of the higher plants (see above) that have been investigated. In any case Keilin offers no definite suggestion for the various stages involved in the breakdown of more complex substances into carbon dioxide and water, and there is no evidence available that oxidation by the cytochrome system leads to the formation of carbon dioxide.

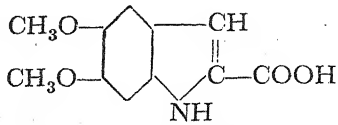
Tyrosinase.—This enzyme oxidizes monohydric phenols, as well as their derivatives, such as the amino-acid tyrosine, to the black pigment melanin. It has been found to be present in many fungi, such as Russula, as well as in the peripheral regions of the potato tuber near the skin and in wheat bran.

The oxidation of tyrosine by tyrosinase has been fully examined by Raper.* The first visible product is a red pigment. This, however, is an unstable body and undergoes spontaneous change into a colourless derivative, and this colourless substance is oxidized by atmospheric oxygen to the black pigment melanin.

Raper has made a full investigation into the series of reactions that occur when tyrosine is converted into the red pigment. He found that when the red pigment was allowed to decolourize in an atmosphere of carbon dioxide, or *in vacuo*, it could be methylated with methyl sulphate. After methylation he was able to

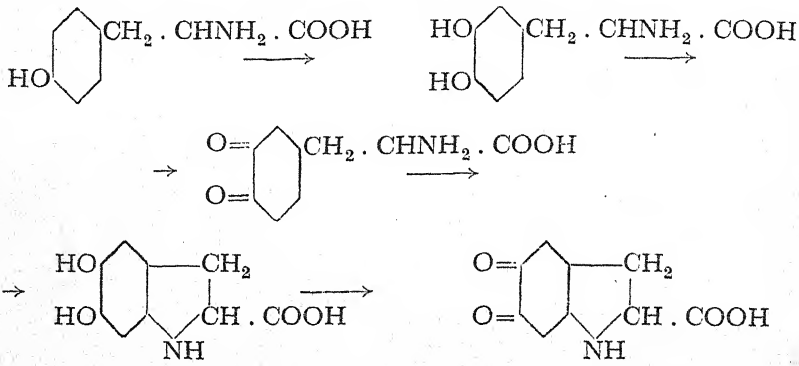
* *Biochem. J.*, 1927, **21**, 89.

isolate two crystalline bodies, one an acid and one a feeble base. These products were discovered to be indole derivatives and the acid was found to have the following structure:



5 : 6-dimethyl-indole-2-carboxylic acid

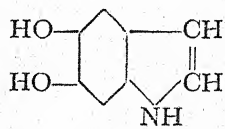
Raper considers that the following series of reactions takes place in the conversion of tyrosine into the red pigment:



5 : 6-dihydroxydihydroindole-2-carboxylic acid

5 : 6-quinone dihydroxydihydroindole-2-carboxylic acid (red pigment)

When the red pigment suffers auto-reduction to the colourless body with loss of carbon dioxide, the colourless product has the structure:



5 : 6-dihydroxyindole

This body in the presence of free oxygen is oxidized to melanin.

Catalase.—This enzyme has a wide distribution in the plant and animal kingdom and decomposes hydrogen peroxide into water and oxygen.

The function of catalase in respiration is not known. In many cases it appears to exert a protective action. For example, when the purine bases are oxidized by the xanthine oxidase, the oxidase

is slowly destroyed by the accumulation of hydrogen peroxide. When catalase is present the hydrogen peroxide thus formed is decomposed and the xanthine oxidase is unharmed.

Carboxylase and Zymase.—The function of these enzymes has already been considered under alcoholic fermentation and will not be further discussed here.

THE MECHANISM OF AEROBIC RESPIRATION

The equation $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$ which is used to represent the respiration of carbohydrate, merely indicates the initial and end products of the reaction. It has already been stated that at ordinary temperatures and in the presence of oxygen glucose does not undergo oxidation to any measurable extent, so that various and special mechanisms must be present in the cell to bring about this breakdown of metabolites.

In 1875 Pflüger observed that frogs kept under anaerobic conditions respired for a time. Impressed by this observation, he suggested that anaerobic respiration is not a pathological phenomenon or biological adaptation to fresh conditions, but a phase in normal aerobic respiration. He considered that during anaerobic respiration easily oxidized substances are formed and these are attacked and further broken down to carbon dioxide and water by atmospheric oxygen.

Pflüger's suggestion was adopted by Pfeffer in 1878 and Wortmann in 1880 for plants, but as alcohol is formed in anaerobic respiration of plants as in the fermentation of sugar by yeast, this substance must be taken into consideration. Pfeffer considered that alcohol is an intermediate product of respiration and is entirely oxidized in air, whereas Wortmann thought that the alcohol is used in the synthesis of sugar.

Pfeffer's views on the genetic connection between anaerobic and aerobic respiration met with considerable opposition. On Pfeffer's hypothesis the ratio between the intensities of anaerobic and aerobic respiration should be constant, and this was not found to be the case. It was, for example, shown by Diakanov that various fungi only respire anaerobically when fed with sugar, and respiration stops completely if other sources of carbon are used, although these substances are utilized by the fungi in aerobic respiration.

On account of these objections Pfeffer himself abandoned his

theory, but later investigations have shown that his original view was fundamentally correct. The objection raised that the ratio of intensity of anaerobic and aerobic respiration should bear a constant relation is more apparent than real. There is no reason why the ratio should be constant. The rate of anaerobic respiration will depend initially on the concentration of respirable substrate and if alcohol accumulates this will slow down the process by exerting a toxic action on the anaerobically respiring cells. On the other hand, in cells respiring under aerobic conditions, alcohol formed in the process will be oxidized to carbon dioxide and water, and therefore will be unable to exert any toxic effect and the rate of the process will not be slowed up.

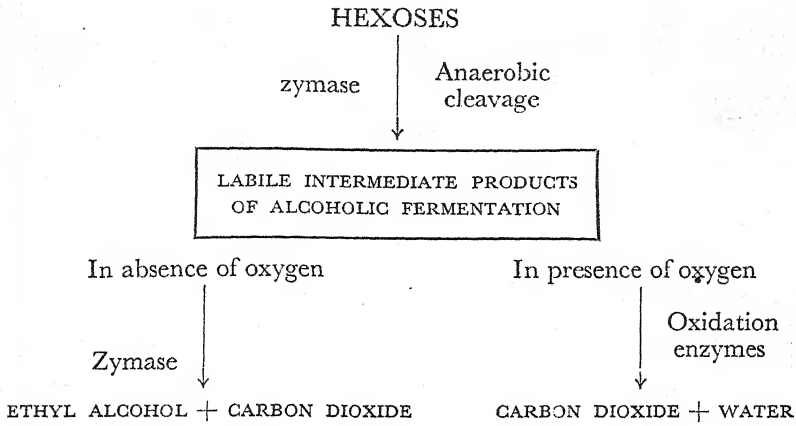
The second objection to the acceptance of Pfeffer's theory was founded on Diakanov's work on fungi and this has been shown by Kostytchev to be due to faulty technique. Kostytchev showed that Diakanov's results were largely nullified by the accumulation of toxic products formed under the conditions of anaerobic respiration. When the toxic conditions were eliminated, no difference was found to exist between the utilization of sugar and non-sugar compounds, such as lactic acid, mannitol and glycerol as substrates for anaerobic respiration.

It is now generally accepted that normal aerobic respiration includes an anaerobic phase. It has already been seen that it is more than probable that acetaldehyde forms an intermediate product of alcoholic fermentation. It was shown by Klein and Pirschle* that acetaldehyde is formed under aerobic conditions in strongly respiring organs such as buds and seedlings, while Bodnar† and others have shown that it is produced in peas grown under anaerobic conditions. M. Thomas‡ has also shown that, in the absence of oxygen, stored apples produce both ethyl alcohol and acetaldehyde, although neither are formed as intermediate or end-products of normal respiration.

The isolation of zymase not only from yeast but from the cells of the higher plants showed the way for modifications in Pfeffer's original views, and Kostytchev has been especially prominent in advocating this view. According to this scheme aerobic respiration proceeds in stages, and it is not ethyl alcohol that is formed but some labile intermediate products of zymase

* *Biochem. Zeit.*, 1926, **168**, 340.
† Ibid., 1925, **165**, 16.
‡ *Biochem. J.*, 1925, **19**, 927.

activity, for one great difficulty in accepting Pfeffer's theory is the fact that ethyl alcohol is a substance which is not readily oxidized. The subsequent fate of these substances depends on the presence or absence of oxygen. Kostytchev's hypothesis is shown schematically below:



F. F. Blackman* has extended the views of Kostytchev in this connection to explain the results of respiration experiments carried out on stored apples by Parija at the Cambridge school of botany.

It was found by Parija† that the respiration of Bramley's seedling apple followed different courses in air and nitrogen. In stored apples a steady drift of respiration with time was observed. Under storage conditions Blackman holds that there is a fundamental change in the organization of the tissues, which allows hydrolysis to proceed at a faster rate than when the fruit is maturing owing to the weakening of the control of protoplasm over hydrolysis. As a result of this increase in the rate of hydrolysis, a larger amount of substrate for respiration is formed and there is an increased formation of carbon dioxide. When this phase ends, there is a decrease in the rate of respiration towards zero from the natural starvation condition that is bound to exist in an isolated plant such as a stored apple.

It was further ascertained that the observed rate of respiration of a stored apple is due to the integration of two independent and opposed processes that are taking place during the senescent phase of the apple, i.e. after the fruit has been picked and stored.

* *Proc. Roy. Soc.* (*Lond.*), 1928, **103B**, 412, 491. † Ibid., 1928, **103B**, 446.

In the first place there is the starvation drift with time which has been described above, and this will naturally tend to lower the rate of respiration; and in the second place there is an acceleration of respiration owing to the increase in the rate of hydrolysis of the substrate for respiration.

It was discovered that when the apples were placed for a long time in an atmosphere of nitrogen, no disturbing effect of a permanent nature occurred in metabolism. The rate of respiration was found to return to that level of intensity which it would have attained if the material had been allowed to remain under aerobic conditions for the same period of time. A characteristic feature that was always found when the apples were transferred from aerobic conditions to an atmosphere of nitrogen was an initial increase in the amount of carbon dioxide evolved, and this value then fell slowly to a level which might be that of the rate in air, and after a time it descended below this line of normal respiration. In some cases, however, after the initial outburst of carbon dioxide in a nitrogen atmosphere, the curve fell as before, but always remained above the air-line level. In the case where the curve fell below the air-line level in nitrogen, it was found that on re-exposure to air the respiration curve fluctuated and eventually reached the air-line level. In the second case, where the curve remained above the air-line level in nitrogen, the curve fell below the normal level of respiration when the apple was replaced in air, and this in turn was followed by a rise until the normal rate in air was attained.

From the data obtained in this investigation, Blackman has put forward a scheme of a general nature for the respiratory process, which is in harmony with the investigations of Neuberg and Meyerhof. In the Blackman scheme there is a chain of four consecutive phases. The first phase is the hydrolysis of reserve carbohydrates, such as cane sugar and starch, to free normal hexoses. In the second phase there is "activation" of these normal hexoses to "heterohexoses with the less stable type of internal ring structure." Presumably these heterohexoses are the active γ-sugars (see Chapter VIII). Following upon this phase of activation comes glycolysis which is brought about by various enzymes of the zymase complex with the formation of intermediate products, such as pyruvic acid, acetaldehyde and possibly lactic acid. The fourth and last phase is respiration in the narrow sense and the nature of the final products formed depends upon

the presence or absence of oxygen. In oxygen, carbon dioxide and water are produced, while under anaerobic conditions ethyl alcohol and carbon dioxide are the end products. It will be remembered that the amount of carbon dioxide produced in aerobic respiration is less than the carbon dioxide formed under anaerobic conditions, i.e. in the presence of nitrogen there is an increase in the carbon dioxide output. In fact it was found in the course of these investigations that the loss of carbon was three or four times as great in nitrogen as in air.

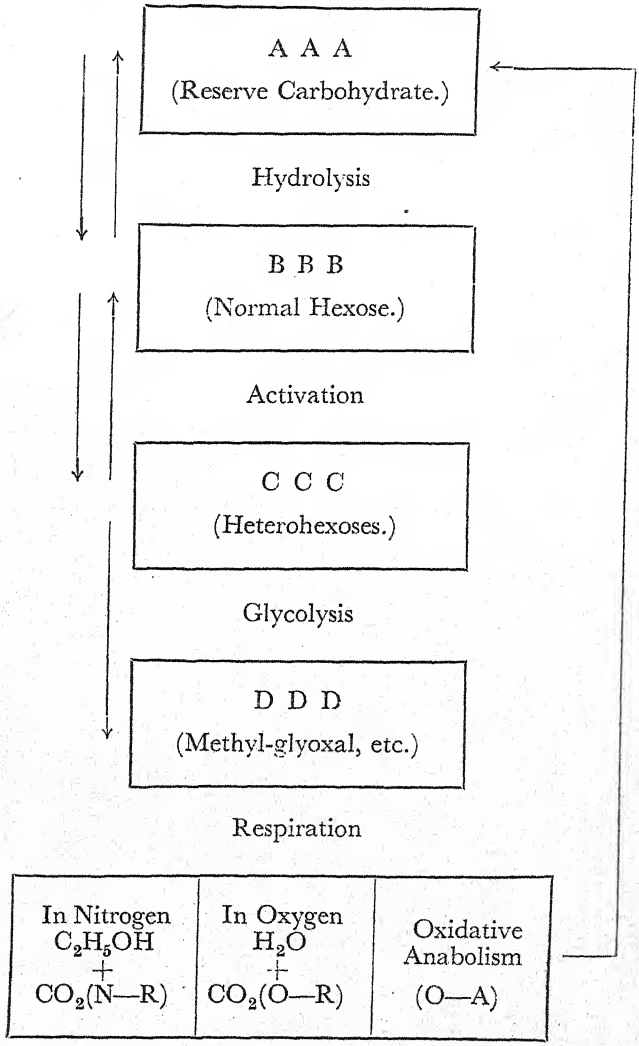
It is also known that in normal aerobic respiration the intermediate products of glycolysis do not accumulate. It has therefore been suggested by Blackman that under aerobic conditions another reactive mechanism is in play that in some way builds back into the system some of the products of glycolysis. This has been termed *oxidative anabolism*. This anabolic re-synthesis is thought to be specific in the presence of oxygen. The first stage of hydrolysis of reserve carbohydrates, as well as activation of normal hexoses, is held to be reversible, but the phase of glycolysis is considered to be irreversible. The Blackman scheme is shown diagrammatically on p. 386.

The view that fermentative cleavage of carbohydrate precedes oxidation in aerobic respiration in all plants has been called in question within recent times by the investigations of Boysen Jensen,* Müller,† and Lundsgaard.‡ It was shown by Boysen Jensen that in Sinapis seedlings and the leaves of Tropaeolum, as well as the two fungi *Aspergillus niger* and *Penicillium glaucum*, the ratio of the rate of respiration under anaerobic conditions to the rate under normal aerobic conditions falls below 1/3 without injury to the respiring material. When one molecule of hexose is oxidized to completion, six molecules of carbon dioxide are obtained, whereas in fermentation, only two molecules of carbon dioxide are formed for every molecule of hexose utilized. Anaerobic processes, therefore, do not decompose sufficient sugar to account for the whole of aerobic respiration.

It has been shown by Lundsgaard that sodium iodoacetate completely inhibits the activity of zymase, and when baker's yeast is treated with this substance in the proper concentration, fermentative activity is brought to a standstill. Sugar, however,

* *Kgl. Danske Videnskabernes Selskab. Biol. Med.*, 1923, **1**, 34.
† *Den. Kgl. Veterinaer, Land. Aarskrift*, 1925, 329; *Biochem. Zeit.*, 1928, **199**, 136.
‡ *Biochem. Zeit.*, 1930, **220**, 1.

is still oxidized to carbon dioxide and water. Evidently, therefore, zymase is not necessary for the chain of reactions in which sugar is completely decomposed to carbon dioxide and water.



Müller has described the preparation from *Aspergillus niger* of a glucose oxidase which is able to oxidize glucose to gluconic acid directly without the intervention of zymase. Here, again, the preliminary breaking down of hexose does not need the presence

of zymase. *Saccharomyces marxianus* and *S. exiguus* apparently do not contain the enzyme maltase and are unable to ferment maltose. Nevertheless, when grown on a maltose medium they are able to absorb oxygen and evolve carbon dioxide. It has been assumed that these forms contain a maltose-oxidase which is able directly to oxidize maltose.

It is evident that we do possess at the present time a certain amount of fairly strong evidence that carbohydrates can be oxidized through the activity of oxidase enzymes that are able to bring about the direct oxidation of carbohydrates without zymase-cleavage. It still remains to be discovered whether under normal conditions the oxidation of the labile intermediate products of glycolysis cannot also take place.

SPECIAL CASES OF RESPIRATION IN LOWER PLANTS

Among the bacteria occur forms which are able to oxidize inorganic material, and there appears to be no respiration of organic compounds. This type of oxidation may be classed as respiration, for the energy released by these oxidative processes is used to carry out the vital activities of these plants.

The nitrifying bacteria, Nitrosomonas and Nitrococcus, which oxidize ammonia to nitrites:

$$2NH_3 + 3O_2 = 2HNO_2 + 2H_2O$$

are among some of the best known of these forms.

The oxidation of nitrites to nitrates is effected through another bacterium, Nitrobacter:

$$2HNO_2 + O_2 = 2HNO_3$$

The function of these forms in the nitrogen-cycle of nature has already been discussed in Chapter X, and it will be recalled that they are able to synthesize carbohydrates from carbon dioxide without the necessity of chlorophyll and the presence of light. The energy for this process is obtained from their oxidative activities in the conversion of ammonia into nitrites (Nitrosomonas and Nitrococcus) or nitrites into nitrates in the case of Nitrobacter.

A number of bacteria are known which are able to oxidize sulphur or its compounds. From the biochemical standpoint these oxidations fall into three groups. In the first group, sulphuretted hydrogen (hydrogen sulphide) is oxidized to sulphur.

This is an intra-cellular process, the sulphur being deposited inside the cells, and when the supply of hydrogen sulphide falls off, this sulphur is oxidized to sulphate. The second group of sulphur bacteria is able to oxidize hydrogen sulphide and also thiosulphates and tetrathionates, but here the sulphur is deposited outside and not within the cell. The third and last group is able to oxidize thiosulphates and elementary sulphur to sulphuric acid and is able to withstand very acid conditions.

The first group, which includes the forms Beggiota and Thiothrix, occur in thermal springs containing hydrogen sulphide. The metabolism of Beggiota has been largely elucidated by Winogradsky. This is a colourless, filamentous form, which can be seen to be filled with grains of sulphur. It was shown by Winogradsky that in the presence of hydrogen sulphide granules of sulphur are deposited in the cells. When the supply of hydrogen sulphide falls off, the sulphur in the cells is oxidized to sulphate which appears in the culture medium. The organisms die off after the disappearance of the sulphur.

The behaviour of Beggiota in water containing hydrogen sulphide can be readily observed under the microscope. The bacteria are in a state of active motion, and as they approach the edge of the cover slip they obtain a supply of oxygen from the air and then return to oxidize hydrogen sulphide in the middle of the preparation.

Beggiota and Thiothrix are chemosynthetic forms and are able to assimilate carbon dioxide. Their development can take place in the absence of organic material.

The equations for the oxidation reactions of these forms may be written:

$$2H_2S + O_2 = 2S + 2H_2O$$
$$2S + 3O_2 + 2H_2O = 2H_2SO_4$$

The second group of sulphur bacteria oxidize hydrogen sulphide and deposit free sulphur outside the cell. The best known form is *Thiobacillus thioparus*. This form not only oxidizes hydrogen sulphide but also thiosulphates and tetrathionates. Like Beggiota and Thiothrix it is able to assimilate carbon dioxide by chemosynthesis.

The equation for the oxidation of thiosulphate by this form is:

$$6K_2S_2O_3 + 5O_2 = 4K_2SO_4 + 2K_2S_4O_6$$

It is not known at present whether the deposition of sulphur is due to direct bacterial oxidation, or whether the precipitation of sulphur is brought about through the interaction of the various sulphur compounds formed.

The third group of these sulphur bacteria was isolated from soils containing free sulphur and rock phosphates by Waksman and Joffe.* This form has been called *Thiobacillus thio-oxidans*. It is an aerobic form and autotrophic, carbon dioxide being the sole source of carbon. It obtains its energy requirements by the oxidation of sulphur or thiosulphates, which are directly oxidized to sulphate. It shows a remarkable tolerance to the presence of free sulphuric acid which it produces, and although its optimum pH is between 3 and 4, it can survive a pH as low as 0·6.

$$2S + 3O_2 + 2H_2O = 2H_2SO_4$$
$$Na_2S_2O_3 + 2O_2 + H_2O = Na_2SO_4 + H_2SO_4$$

The iron bacteria when grown on media containing iron salts form on their outer sheath a deposit which consists principally of ferric hydroxide. Two forms must be briefly considered here, *Leptothrix ochracea* and *Spirophyllum ferrugineum*. The latter form is strictly autotrophic and obtains its supplies of carbon from carbon dioxide; there is some doubt, however, as to whether *L. ochracea* is autotrophic. It is supposed that these bacteria obtain their energy requirements by the oxidation of ferrous to ferric iron:

$$2Fe(HCO_3)_2 + O + H_2O = 2Fe(OH)_3 + 4CO_2$$

The hydrogen bacteria, which are soil forms, oxidize hydrogen to water, and from this reaction obtain energy for the assimilation of carbon dioxide without a supply of radiant energy:

$$2H_2 + O_2 = H_2O$$

The best known of these hydrogen oxidizers is *Bacillus pantotrophus*.

* *J. Bact.*, 1922, 7, 239.

PART III

GROWTH, REPRODUCTION AND IRRITABILITY

CHAPTER XIV

GERMINATION

THE germination of seeds and spores is a large subject, and the conditions for germination of the seeds of different plants vary widely. The seed of a higher plant is a very specialized structure and consists in the vast majority of cases of a resting embryo which possesses first leaves or cotyledons, a radicle, and plumule, which eventually develops into stem and leaves. In most seeds, the plumule is an extremely small conicle structure, showing no trace of young leaves. In other seeds, however, such as the Almond, it is large and bears small outgrowths which can be recognized as undeveloped leaves. Seeds are also supplied with a store of reserve food material, which is to be found either in the cotyledons or in a special structure, the endosperm. The water-content of seeds is low, in some cases as low as 10 per cent.

It will be seen that the seed of the flowering plant is an organ admirably constructed for the purpose of withstanding a period of adverse conditions, for with its low water-content and no sap in the vacuoles, chemical reactions are reduced to a minimum and respiration is very slow. With the advent of suitable conditions for germination, the newly-developing plant draws upon the reserve food supplies in cotyledons or endosperm in the preliminary stages of its growth. On the other hand, the spore of the lower plants is not a specialized structure like the seed. It has no resting embryo and cannot withstand prolonged periods of rest or of adverse conditions. Fungus spores appear to be more resistant in this respect than those of other plants. Ramsbottom,* for example, has described the germination of fungus spores which were at least fifty years old.

Conditions of Germination.—The principal factors for the successful germination of seeds are: (1) Water, (2) Oxygen, (3) Temperature and (4) Light.

For successful germination to take place, water is necessary. Different seeds show a wide variability in the amount of water they take up as a preliminary to germination. The wheat grain absorbs between 40 to 60 per cent of its dry-weight, while the

* *Handbook of the Larger Fungi*, Brit. Mus., Lond., 1923.

pea takes up between 84 to 106 per cent and the maize between 35 and 40 per cent.

The initial stages of water absorption by a seed are due to the process of imbibition by the colloids of the embryo, endosperm and seed coats. Later, cell vacuoles are formed, and osmotic forces come into play.

Whether the preliminary soaking of seeds in water improves their powers of germination has always been a matter of controversy. This question has been very thoroughly investigated by Morinaga,* who worked with some 78 different genera, and found that in 43 of the 78 genera investigated germination could take place under water. In only two cases did he find that germination took place better under water than on damp filter paper, while in 18 cases germination under water was equal to that on damp filter paper. He was able to show that if the oxygen-content of the water were increased, seeds of several genera which refused to germinate under water, germinated easily.

In the absence of oxygen germination fails to occur. It has been shown by Morinaga that the seeds of *Typha latifolia* are peculiar in this respect. He found that he could obtain better germination if the pressure of the oxygen of the air is reduced by partial replacement with either nitrogen or hydrogen.

Temperature plays several different rôles in germination. In the first place it affects the rate of entry of water into the seed as well as the growth rate. In the second place it affects the resistance offered by the seed coats to the extrusion of the radicle.

The minimum, maximum and optimum temperatures of germination of different seeds have been determined by several investigators. The bean (*Phaseolus vulgaris*) will show signs of germination at 9° C., and the growth rate increases up to 36° C. and stops at 46° C. Rye will show signs of germination at still lower temperatures (1° C.), the maximum temperature being 30° C. and the "optimum" 25° C. In all cases, investigators have described a minimum, maximum, and optimum temperature for germination. In point of fact, as F. F. Blackman has pointed out, these optimum points are largely illusory, for they will vary with the duration of time over which the temperature has operated. Blackman's concept of the "time-factor" is of importance in this connection, for the temperature may be too

* *Amer. J. Bot.*, 1926, **13**, 126, 141, 159.

high for further growth to take place once the radicle has made its appearance. Thus, although for a period high temperature has brought about rapid growth, the rate falls off owing to the "time-factor" being brought into operation.

Dormancy.—It has already been stated that in the seed with its resting embryo, reserve food supplies and low water-content, the chemical reactions, especially respiration, are reduced to a minimum. In this condition the seed is able in a number of cases to retain its vitality for a long period, up to several years in some cases.

Seeds show a wide variation in their power of withstanding long periods of keeping. Oxalis probably exhibits the shortest period. The seeds germinate as soon as they leave the capsule, and quickly die if exposed to a dry atmosphere. Similarly the seeds of willow (Salix) are only viable for a few days. At the other end of the scale we have wheat, which is able to lie dormant for as long as sixteen years, while among the Leguminosae the greatest power of withstanding prolonged periods of keeping has been found, up to 87 years in one exceptional case. The seeds which show the greatest resistance to keeping usually have a tough testa which prevents water absorption when intact and is impervious to the action of gases.

Crocker* has reviewed the physiology of dormancy or delayed germination and attributes this state to one or more of the following causes:

(1) Incomplete development of the embryo.

(2) Impermeability of the testa to water.

(3) Mechanical restraint offered to the expansion of the embryo and other seed contents by the seed coats.

(4) Inhibition and retardation of gases to or from the embryo, resulting in an accumulation of carbon dioxide within the tissues of the embryo, or in an insufficient supply of oxygen for germination.

(5) The necessity for the embryo itself to undergo certain after-ripening processes before germination and growth under ordinary conditions becomes possible.

(6) Introduction by various means of a condition of dormancy in seeds previously capable of immediate germination.

The term "secondary dormancy" has been applied by Crocker to (6).

* *Amer. J. Bot.*, 1916, 3, 99.

Among the Gymnosperms, Gnetum and Ceratozamia furnish examples of seeds in which the embryos are not completely developed and continue to grow after the seed has fallen. *Ginkgo biloba* shows a very plastic condition in this connection. In Japan, after fertilization has taken place, the embryo may or may not be mature. In plants grown in Europe, fertilization does not take place until after the seed has fallen. In some dicotyledons such as the Lesser Celandine (*Ranunculus Ficaria*), the embryo is small and undifferentiated when the seed is shed, and only develops slowly through the autumn and winter and is ready for germination in the spring. The seeds of orchids are minute and produced in enormous numbers, and contain an undifferentiated embryo. The seeds of these plants will only develop under natural conditions in the presence of a fungus (see under Mycorrhiza).

It has been shown by Rose* that the freshly-harvested seeds of *Tilia americana*, which have a water-content of 10 per cent or even less, fail to germinate when placed on a moist substratum at ordinary temperatures. Germination also failed to occur if the seeds had been kept at warm temperatures for several months. Before successful germination of seeds of *T. americana* can be brought about, they must be submitted to a period of after-ripening, which includes a period at 0° C. to 2° C. under moist conditions for a certain time. This period of low temperature must be followed by another period of two or three weeks at 10° C. to 12° C., when germination commences, and if successful growth of the seedlings is to take place, a still higher temperature must be employed.

It was also shown by Rose that the seeds of *Sambucus canadensis* will not germinate unless they are first kept out of doors in moist soil for the length of the winter. The reason for this behaviour was not discovered.

The seeds of Juniperus must be kept for 100 days at a temperature of 5° C. before they will germinate. During this time the embryo undergoes a process of after-ripening. The following changes were found by Pack† to occur during this period of after-ripening. There is a rise in the pH of the embryo, an increase in titratable acid, decrease in stored fats and proteins and increase in sugar-content. At the same time fat is translocated from the endosperm to the embryo, the respiration rate of the embryo shows a slight rise and there is a marked increase in catalase activity.

* *Bot. Gaz.*, 1919, **67**, 281.

† Ibid., 1921, **71**, 32.

The seeds of *Cornus florida* are dormant when the fruit has first matured, and this dormancy cannot be broken by treatment with acids, ether or ethylene. A period of after-ripening of from 100 to 130 days is necessary at temperatures between 0° C. to 10° C. before germination will take place. It was found by Davis* that although there is an increase in starch-, sugar- and amino-acid-content in the seeds during the period of after-ripening, these cannot be correlated with the after-ripening process, whereas catalase activity showed a remarkable parallel to this process. He has suggested that the after-ripening process is closely linked with respiration, mainly on the grounds that respiration falls until germination actually begins in spite of the increase in potential respirable substrates, e.g. starch and sugars, while at the same time there is an increase in catalase activity; whereas if the seeds were exposed to higher temperatures (15° C. and over) there is an increase in respiratory substrate, a rise in respiration rate and a fall in catalase activity.

Seed and fruit coats can inhibit germination in a number of ways. Thus the coats may prevent absorption of water, or the intake of oxygen, or may even act by mere mechanical restraint. With regard to this last factor, it has been shown by Rose† that dormancy in *Rubus Idaeus* is due to the high breaking strength of the endocarp. The marine pea, *Lathyrus maritimus*, possesses a particularly tough testa, and according to Stiles and Dellow‡ will not absorb water even after immersion for a year, nor will the seed germinate when planted out. Before germination will take place, the testa must be ruptured. The suggestion has been put forward that under natural conditions this plant either propagates itself by means of vegetable suckers, or that the seeds are cast on the shingle and cracked by the action of waves on the pebbles.

The effect of light on germination has been much investigated during recent times. It has been known for many years that the seeds of some plants will only germinate in the presence of light, whereas in other cases germination will only take place in the dark.

Tobacco occupies a somewhat intermediate position in this connection. The seeds germinate more readily in light than in darkness, but darkness does not completely inhibit germination; it merely retards it. The seeds of *Aquilegia atrata* can be kept on

* *Bot. Gaz.*, 1927, **84**, 225. † Loc. cit. ‡ *Ann. Bot.*, 1924, **38**, 209.

a moist substratum in darkness for as long as 10 years, and only a small percentage will germinate, but on exposure to light germination takes place readily. These examples of seeds which will only germinate in the light can be multiplied indefinitely, but the reason for this behaviour is not known. It has been suggested by Lehmann* that in "light" seeds the action of light is to catalize the hydrolysis of reserve proteins into soluble compounds. The light is supposed in some way to activate the proteolytic enzymes. Evidence in support of this view is the fact that extremely small exposures to light are sufficient to bring about germination. As brief an exposure as 1/10 second with a light intensity of 730 candle-power at a distance of 1 metre, is sufficient to initiate germination in *Lythrum salicaria*. On the other hand, in "dark" seeds, light is considered to activate fluorescent organic compounds which have a destructive effect upon the proteolytic enzymes present. It is very doubtful, however, whether this explanation is correct, for the physiological conditions involved are undoubtedly very complex.

The condition of "secondary dormancy" has been induced in the seeds of *Brassica alba* by maintaining them in an atmosphere of carbon dioxide. Kidd and West† found that under these conditions germination is completely inhibited, and this inhibition of germination can be enforced for as long a period as twelve months. After exposure to carbon dioxide, the seeds refuse to germinate until the testas be removed or the seeds dried. The carbon dioxide apparently has some kind of stabilizing action on the embryo of the seed, for if the testas be removed with extreme care, the naked embryo will still remain in the dormant condition, and while in this state the embryos do not respond to ordinary environmental conditions which govern the course of germination of normal embryos. Some kind of stimulus, either mechanical or chemical is necessary to initiate cell division and therefore growth of the dormant embryo.

MYCORRHIZA

In certain families of plants, notably the Orchidaceae and the Ericaceae, germination of the seeds will not normally take place except in the presence of a fungus. Unless the seeds of an orchid come into contact with its suitable fungus, no or very little germination will take place. It has been long known among

* *Ber. deut. bot. Ges.*, 1918, **36**, 157.

† *Ann. Bot.*, 1917, **31**, 456.

orchid growers that it is a difficult matter to get orchid seeds to germinate, and it used to be the old horticultural practice to sow the seeds upon soil in which orchids had been growing. When this procedure was adopted fairly good germination was obtained, the reason being that the mycelium of the fungus was present in the soil and could infect the seeds, although, of course, this fact was not known to the orchid cultivators, who had merely hit upon this discovery accidentally.

The term *mycorrhiza* was coined by Frank for this association of higher plant and fungus. Mycorrhizal association is of wide occurrence in the plant world and has been much investigated. It finds commercial application in the cultivation of orchids.

The conditions governing the germination of orchid seeds were very fully investigated by Bernard, who was able to remove many of the difficulties associated with the germination of the seeds of these plants. The number of seeds produced per capsule in orchids is enormous. The seeds themselves are minute dust-like particles each containing an undifferentiated embryo. When sown in the ordinary way, the seeds are sterile, but when the necessary fungus is present, germination proceeds smoothly and normally. The fungus concerned here belongs to the genus Rhizoctonia.

In certain cases, even in the absence of fungus, germination will actually commence. For example, in *Bletilla hyacinthia*, in the absence of the fungus germination will proceed as far as the first leaf stage, but the seedling does not survive beyond this point. In the presence of the fungus germination takes the following course. The fungus enters the seed at the suspensor end, and the invasion of the cells takes place by degrees; the hyphae become twisted into a ball in each cell before passing on to the next. In some way which is not known at present, the presence of the fungus stimulates the smaller cells at the end of the seed opposite the suspensor, and these now proceed to divide. The stem meristem is laid down in this region, and it should be observed that the fungus never penetrates the meristematic cells. The seed now becomes swollen in shape and the later stages of development are carried through.

Bernard attempted to germinate orchid seeds in the absence of their endophyte and employed solutions of cane sugar and salep of varying concentration for this purpose. He found with Bletilla, Laelia, and Cattleya that with certain concentrations there was

germination and in higher concentrations seedlings were obtained which could be transplanted. More recently Knudson* has made a number of observations on this matter and considerably extended this aspect of the subject. He showed that with the seeds of a hybrid orchid (*Cattleya Schraederae* × *C. gigas*), which had previously been carefully sterilized in a weak solution of calcium hypochlorite and sown on either Pfeffer's medium or a modification of this in 1·7 per cent agar, good germination occurred in the presence of fructose, but that with glucose the seedlings tended to show chlorosis. He also showed that plant extracts are able to answer this purpose. This work was later extended by him to other species.

Knudson next found that germination could take place in the presence of the fungus on a medium containing starch and that its presence accelerated germination on Pfeffer's medium and cane sugar. He was able to show that one of the most important features controlling germination of orchid seeds was the concentration of starch in the external medium. The fungus completely hydrolysed the starch to soluble sugars and the pH of the medium became altered from an unfavourable to a favourable reaction. Further, it was shown that germination took place in the presence of the fungus, although the seeds were not penetrated. This was found to be due to the change in the pH of the medium. Lastly, Knudson was able to bring about germination in a medium composed of a mixture of peat and sphagnum plus Pfeffer's modified medium, provided that the pH was adjusted to 4·6. In these circumstances germination was just as rapid as in the presence of the endophyte. Moreover, Knudson ascertained that Phytophthora (sp.) as well as other fungi are just as efficient in bringing about normal germination of orchid seeds as the true endophyte Rhizoctonia. It will be convenient to postpone any consideration of Knudson's theoretical views of this matter until the question of the germination of ericaceous seeds has been described.

The first adequate investigations on the mycorrhizal relations of the Ericaceae were carried out by Ternetz, who was able to isolate the endophyte and showed that it was a member of the Fungi Imperfecti. She was also able to show that the infection of the seedling of the common ling, *Calluna vulgaris*, occurred through the seed.

* *Bot. Gaz.*, 1922, **73**, 1; 1924, **77**, 212; 1925, **79**, 345.

Rayner* has made a large number of investigations on the mycorrhiza of the Ericaceae. In the first place she claimed to show that germination of the seeds of *Calluna vulgaris* is absolutely dependent on the presence of the endophyte, and that there is an obligate symbiosis present of a very similar type to that of the Orchidaceae. In the Orchidaceae, however, the endophyte is kept strictly confined to the root system in the after life of the plant, but in Calluna, according to Rayner, the endophyte is said to be present through the whole plant, and infection of the seeds takes place in the ovary. A remarkable condition of affairs.

Rayner found that when the seeds of *C. vulgaris* were sterilized in weak mercuric chloride solution, only feeble germination took place. No complete root system was developed by the seedling, only a few chlorotic leaves were formed and the seedling eventually died. If, however, at this stage of development the seedling was supplied with the endophyte, development proceeded normally. Infection of the seedling root took place immediately after it emerged from the testa, and the entering hyphae forced their way in between the cells of the root apex. The mycelium now became intracellular and rapidly spread from cell to cell. Eventually it was distributed through the length of the plant and was to be found in stem, root, leaves and reproductive organs.

This work of Rayner has been criticized by Christoph,† who claimed that there is no necessity for the root-fungus for germination in the Ericaceae. He tested the matter in two ways. In the first series of experiments, cuttings of *Calluna vulgaris* were planted in shallow pots filled with humus heath soil, and the soil in one series was sterilized and the other not. In both series a number of seedlings "struck," and it was only cuttings from unsterilized soil that showed slight fungal infection, whereas no endophyte was present in the roots of the cuttings growing on sterilized soil. Similar results were obtained with *Erica carnea*. Germination experiments with sterilized and unsterilized seeds also gave good results in both cases and Christoph failed to find capsule infection.

In the allied family of the Pyrolaceae, Christoph found that the fungus present in *Pyrola uniflora*, *P. secunda*, *P. minor*, and

* *Ann. Bot.*, 1915, **29**, 97; *New Phyt.*, 1916, **15**, 161; *Bot. Gaz.*, 1922, **73**, 226; *Trans. Brit. Myc. Soc.*, 1922, **8**, 61; *Ann. Bot.*, 1929, **43**, 55.

† *Beihft. Bot. Centralbl.*, 1921, **38**, 115.

P. rotundifolia possessed clamp-connections, and therefore was probably a Basidiomycete and not a Phoma, as the endophyte of the Ericaceae has been claimed to be. Here, again, he found that the presence of the fungus is unnecessary for germination, and that the best conditions were obtained when strong concentrated soil solutions were used, together with the addition of a peptone solution, sowing on humus from the habitat of the plants, and keeping the cultures in the dark with moderate moisture.

According to Christoph, the Ericales present a case of facultative and not obligate symbiosis. Rayner* has submitted this work to a detailed criticism, but the burden of her argument is that Christoph failed to sterilize his material sufficiently thoroughly. Knudson,† however, has presented a number of facts in this connection which do not support Rayner's remarkable results.

Knudson has suggested that the abnormal appearance shown by Rayner's seedlings in the absence of the endophyte were due to the fact that the nutrient solution in which the seedlings were grown was toxic to them, or alternatively, that the use of mercuric chloride as a sterilizing agent led to injury of the embryo. He has carried out a detailed reinvestigation of Rayner's work using the same conditions as those he employed for the germination of orchid seeds. Rayner's nutrient medium was used with the addition of 1·5 per cent of agar, and in certain cases 2 per cent of glucose was added to the cultures.

The seeds were sterilized in calcium hypochlorite solution in place of the mercuric chloride used by Rayner, and in the first set of experiments no attempt was made to separate the seeds from the floral tissues. It was found that all the tubes showed infection, but the fungus proved to be a species of Altenaria and not the endophyte of the Ericales, which is stated to be a species of Phoma. Thus far this work shows that the seeds are not infected in the ovaries as Rayner claimed. The growth of the seedlings was found to be erratic; the best seedlings that were selected for further growth showed no infection of the roots, and in all cases the roots were perfectly healthy.

In a second set of experiments, the seeds were carefully separated from adhering tissues and sterilized in calcium hypochlorite, and the pH of the culture medium was varied from between 4·5 to 6·6. No optimum pH was found for growth, and

* "Mycorrhiza," *New Phyt.* Reprint, 1927. † *New Phyt.*, 1929, **28**, 369.

no fungal growth was found from the surrounding testa, and the agar was quite free from all fungal contamination. Knudson has suggested that Rayner's results in the absence of the endophyte may well have been due to the presence of excess of iron in her nutrient medium, and that possibly mercuric chloride was too drastic a sterilizing agent.

Rayner* has criticized this work, and again, as in the case of Christoph, the burden of her argument is that the seeds were not properly sterilized and that calcium hypochlorite is not an effective sterilizing agent for Calluna seeds, owing to the nature of the testa and the buoyancy of the seeds. This is by no means a convincing argument, nor is her second contention, that owing to the fineness of the mycelium in its early stages of growth it might have been overlooked, any stronger. In fact it is a little reminiscent of the emperor's dress clothes, and Knudson† has been able to dispose of both of them in a further set of investigations. The seeds were again sterilized with calcium hypochlorite, which was found to be perfectly efficient for this purpose, and grown on a mineral medium containing 1·0 per cent of glucose. When the pH was adjusted to 4·9, good germination was obtained and no contamination was found. When the seeds were sown on a potato-glucose medium, germination took place, but the radicles were short and soon turned brown and died. They presented the same appearance as in mineral culture without the addition of sugar. This result suggests that there was nutritional deficiency of some kind which prevented further growth.

Knudson also used Rayner's own solution. There appears to be a mistake in the original paper as to the composition of this mixture, in which the peptone and glucose content is given as 0·1 per cent, presumably 1·0 per cent was meant. When Knudson employed these lower concentrations he found normal growth to occur with a well-developed root system, but when the concentration of the glucose and peptone was increased to 1·0 per cent, injury to the root was found and there was a marked reduction in the amount of germination. Moreover, he examined the roots of an enormous number of seedlings in an attempt to discover the presence of the endophyte, using the same technique as that described by Rayner and failed to find any trace of mycelium. It would thus appear that infection of the seeds does not take place in the ovary as Rayner originally stated, nor

* *New Phyt.*, 1929, **28**, 377

† Ibid., 1933, **32**, 115.

does the presence of the endophyte appear to be necessary for germination, provided the correct conditions of mineral culture and sugar be present.

The question can be conveniently discussed here of the relationship between endophyte and host in orchids and other mycorrhizal plants. It was originally suggested by Bernard that in orchids this relationship was similar to that of host and parasite. The orchid was supposed to suffer from a "benign cryptogamic disease," and the symbiotic relationship between host and fungus was to him the immunity realized by phagocytosis. On the other hand, it was suggested by Burgeff that the function of the fungus was to hydrolyse polysaccharides to simpler sugars which helped germination, while Knudson had adopted an extreme view of the matter.

Knudson considers that the necessity of the fungus for germination has not been proved, and that germination of orchid seeds, and presumably those of the Ericales as well, is dependent upon a suitable external supply of organic material, such as soluble carbohydrates; and that orchid embryos, when allowed to germinate under aseptic conditions in the absence of sugar, are unable to assimilate because they lack some internal factor. He objects to the idea of the "so-called symbiotic theory of germination," partly on the grounds that the endophyte extracted by himself from Odontoglossum was extremely pathogenic and rapidly killed this plant. To him the great fact for orchid germination is the presence of a supply of soluble sugars, for the orchid seed lacks adequate food reserves, and therefore some external source of supply is necessary to carry the embryo over the critical period until it is in a position to synthesize carbohydrate for itself.

This work of Knudson is really an extension of the early investigations of Bernard, who showed that development would take place in the absence of the endophyte and in the presence of sugars, and that in many cases this method gave more certain results than the use of the fungus. Nevertheless, this work does not explain the fact that under natural conditions, the endophyte is always to be found in the roots of these plants. Ramsbottom* has also criticized Knudson on the ground that the endophyte always penetrates the orchid seed from the suspensor end, and that the cells at the opposite end do not become infected, and

* *Proc. Internat. Cong. Plant. Sci. Ithaca,* 1929.

further, that seeds which adhere to the sides and top of the culture flasks only germinate when the hyphae reach them, coupled with the fact that he has never observed a young rootlet to become infected until it has entered the soil, supports his belief in the symbiotic theory of germination.

Against this must be set the fact that Knudson's most recent investigations on Calluna, which, after Rayner's investigations, was always held to be an extreme example of the necessity of the fungus for germination, have shown that germination will proceed normally in the absence of the endophyte, and that infection does not take place in the ovary. It is really extremely doubtful if the mycelium does penetrate throughout the whole plant, as Rayner has described. Altogether the old theory of symbiotic germination is of very dubious worth for this plant.

GERMINATION OF FUNGAL SPORES

The factors controlling the germination of fungal spores are varied and can only be considered in outline. A large number of parasitic forms can be made to germinate in the presence of water alone. Saprophytic types, such as *Pyronema confluens*, need a nutrient medium and can be grown in an artificial medium composed of inorganic salts and carbohydrates. Others again, and in the main parasitic forms, need some kind of definite stimulus before germination will take place. With *Agaricus campestris* Ferguson* found that no germination took place except in the presence of the mycelium or portion of the fruit of this fungus. On the other hand, Dodge† has ascertained that spores of *Ascobolus Winteri* will not germinate in the presence of the ascocarp.

As a general rule germination can only be brought about in the large section of coprophilous forms (i.e. forms inhabiting dung) by some preliminary treatment leading to the rupture of the spore-coat (epispore). Sordaria, however, will germinate quite readily on rabbit dung decoction without any kind of previous treatment. Under normal conditions, the spores of these dung-inhabiting forms are shot on to the surrounding grass and from here pass through the alimentary canal of herbivorous animals and are ejected in the faeces, when they are capable

* *Bur. Plant Ind. U.S. Dept. Agric., Bull.*, 1902, **16**, 1.
† *Bull. Towey Bot. Club*, 1912, **39**, 139.

of germination. Until they have passed through the alimentary tract they are incapable of germination. Fraser* and others have been able to germinate the spores of several coprophilous Ascomycetes under artificial conditions by a variety of methods. In some cases the spores were incubated for several hours at 38° C. (the temperature of the body of the cow) and then sown on dung decoction, or germination could be brought about by sowing on hot agar after sterilization. According to Ramsbottom, cracking of the epispore by rubbing between cover-slips is sufficient to bring about germination. Thus the various methods described above are merely means to the same end, the cracking of the epispore.

Baden† has shown in *Coprinus sterquilinus* that in addition to warmth and an alkaline medium (aqueous extract of horse dung), the presence of bacteria is necessary for germination. On the other hand, bacteria exert an inhibitory influence on the germination of the spores of Mucor and Rhizopus.

According to W. Brown,‡ oxygen, within wide limits, has very little effect upon the germination of ordinary fruit-rot organisms. Carbon dioxide exerts a definite retarding effect on germination, which becomes more marked with low temperatures and weak nutrient solutions in which the fungal spores have been sown, and in a less degree upon the density in which the spores have been sown. Brown has suggested that the inhibitory action of carbon dioxide is due to the tension set up by this gas in the living plasma, and this tension of gas is considered to be controlled by the pressure of the carbon dioxide in the surrounding atmosphere.

It has also been shown by Brown§ that in the presence of the bruised leaves of Eucalyptus, Choisya and Ruta, the leaves of which contain a number of essential oils, the germination of the spores of *Botrytis cinerea* is facilitated. The amount of germination was determined by measuring the length of germ-tube of a large number of spores chosen at random from a drop of spore suspension and dividing the total length of the germ tubes by the number of spores counted. Even the crushed leaves of *Vicia Faba* will bring about this result. The presence of blotting paper, however, exerted a retarding influence on germination. The reason for this curious result is not known. It appears to give off some volatile substance. Ethyl acetate and ethyl alcohol were

* *Ann. Bot.*, 1907, **21**, 349; *New Phyt.*, 1907, **6**, 156; *Ann. Bot.*, 1909, **23**, 399.
† Ibid., 1915, **29**, 135. ‡ Ibid., 1922, **36**, 257. § Ibid., 1922, **36**, 285.

found to show a distinct optimum effect upon the germination of *B. cinerea*. With ethyl acetate the following figures were recorded:

Control	*Ethyl Acetate*		
	1 *drop*	2 *drops*	10 *drops*
0·36	1·82	1·54	0·46

Although the bruised leaves of Eucalyptus were found to have an encouraging action on germination, yet one drop of Eucalyptus oil in 3 litres of distilled water had a marked retarding effect on spore germination.

Moisture and temperature are important factors in connection with the germination of fungal spores. Among the Phycomycetes moisture is a determining factor of germination. The spores of Venturia, the fungus responsible for the scab disease of pears, will not germinate unless completely immersed in water. As in the germination of seeds, so in the germination of fungal spores, temperature plays an important rôle. It has been claimed that there is an optimum, maximum and minimum temperature for germination. It is probable, however, that the optimum points are fictitious, as is the case for seeds, and that the Blackman time-factor is involved.

Light has in certain cases an important effect upon the germination and after development of many fungi. Cultures of *Phytophthora infestans* and *Pyronema confluens* remain sterile and the uredospores of the Rusts fail to cause infection in the dark.

According to Weiss,* the best conditions for the vigorous development of the potato parasite, *Synchitrium endobioticum*, which is the cause of the so-called wart disease of potatoes, are dependent on the vigorous development of the host. Water is a necessity for the resting and soral sporangia, and there is an indispensable minimum of water necessary for the successful distribution of the zoo-spores. If the soil water-content does not reach saturation, germination is prevented, but if it is constantly near saturation germination is depressed, probably through some adverse reaction on the host plant. The most favourable conditions for infection prohibition, therefore, is periodic flooding, followed by drainage and aeration of the soil. It was found that infection of the host plant could take place over the temperature range of 0° C. to 30° C., but when the soil temperature was maintained at a con-

* *Amer. J. Bot.*, 1925, **12**, 413.

stant value, infection was discovered to be limited to a temperature range of 12° C. to 24° C., but with the variable temperatures present under field conditions, infection takes place at about 21° C., though the upper range may be as high as 30° C. The pH of the soil was also found to have an important influence on infection, and the most favourable range of soil pH for infection was found to be between 3·9 and 8·5.

CHAPTER XV

GROWTH

THAT living organisms grow, i.e. increase in volume, is an obvious phenomenon, but nevertheless it is a difficult matter to define growth. Seedlings, for example, grown in the dark, may be said to grow, for they show increase in size. If, however, the dry-weight of such seedlings be compared with the dry-weight of the seeds, it will be found that there is an actual loss in weight. Grown in the dark in this way, the seedling is unable to assimilate, but respiration, which means the physiological combustion of organic matter, and therefore loss of matter, is continuing the whole time. Thus the seedling shows a loss in dry-weight. If growth be defined as increase in size or volume, then a seedling grown in the dark may be said to have grown, but the increase in volume is largely due to the absorption of water by the cells which have completed division. The formation of new cells by division and their partial differentiation take place in special regions, the meristematic regions of shoot and root and the cambium.

Various methods have been adopted to measure plant growth, such as increase in volume, increase in length, increase in area and increase in dry-weight. These various methods of estimating plant growth have all yielded valuable results, but the only true criterion of growth is increase in dry-weight. The growth of a living organism can only result from successful metabolism, and metabolism has a credit and debit side to be considered. If the credit side (anabolism) be greater than the debit side (catabolism), then there will be excess of material which will be available for the building up of new tissues. On the other hand, if the rate of the catabolic processes be greater than the anabolic ones, the organism will be unable to accumulate excess material for the repair of its tissues or to build up fresh ones, and will sooner or later die.

Some experiments by Boysen Jensen* illustrate this point. He showed that in Sinapis, a sun-loving plant, the maximum intensity of assimilation was 6 mg. of carbon dioxide per hour per 50 cm^2. of leaf surface at 20° C., whereas the rate of respiration

* *Bot. Tidsskrift.*, 1918, **36**, 219.

was found to be 0·8 mg. of carbon dioxide per hour for the same leaf area at the same temperature. Boysen Jensen calculated that the amount of dry-matter assimilated in the course of a day was 60 mg., and the loss in dry-matter from respiration 14 mg. Therefore the gain of dry-matter over loss was 46 mg. or 16·5 per cent. In a shade plant, such as Oxalis, although the assimilation and respiration rates were discovered to be considerably less, an

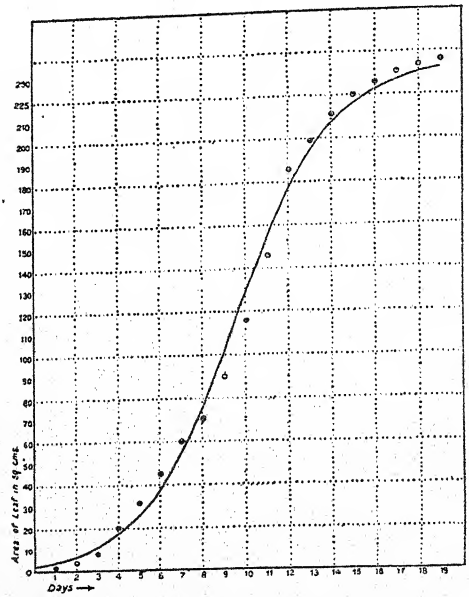


FIG. 35.—S-shaped curve for increase in surface area of leaf of *Cucumis sativus*. (After Gregory.)

excess balance was found of assimilation over respiration. Thus in each case there is a balance of material available for fresh growth.

Plants, as well as their various organs, do not all increase in growth at the same rate throughout the period of their development. Initially the rate is slow, gradually increasing to a maximum and then falling away. Sachs used the expression the *grand period of growth* to designate the time lapse corresponding to this march of rate of enlargement. The curve expressing growth through such a vegetative period is S-shaped. The same S-shaped curve is obtained if the individual organs of a plant be measured in place of the whole plant (Fig. 35).

It has been shown by Gregory* that the increase in length, breadth and area of the leaves of *Cucumis sativus* shows a grand period of growth under normal conditions in full sunlight, while Priestley and Evershed† obtained a sequence of S-shaped J. H. curves for the formation of roots from cuttings of *Tradescantia zebrina*. The time of transition from one curve to the next was found to correspond with the time of appearance of a new crop of roots of a secondary order.

G. E. Briggs, Kidd and West‡ have analysed the large amount

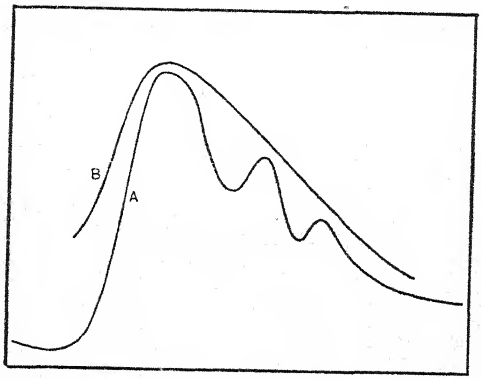


FIG. 36.—A, Growth-rate curve of maize ; B, Leaf-area ratio curve. (From G. E. Briggs, Kidd & West.)

of data obtained by Kreusler and his co-workers on the growth of maize in terms of dry-weight, leaf area and time, and have also employed the measure "relative growth rate" which was defined as the weekly percentage increase in dry-weight plotted against time, and the "leaf area ratio" which was taken as the increase in leaf area in square centimetres plotted against time. The growth rate of maize, using these different criteria, was found to vary in a definite way at different periods in the life history. In the first stage there was found to be an actual decrease in weight, due presumably to losses from respiration. In the next stage there was a rapid period of increase which attained a maximum, and this was followed by a continuous fall. On the falling part of the curve subsidiary maxima made their appearance, and these coincided with the appearance of the male and female inflorescences (Fig. 36).

* *Ann. Bot.*, 1921, **35**, 93.
† Ibid., 1922, **36**, 225.
‡ *Ann. Applied Biol.*, 1920, **7**, 103, 202.

It has been suggested by V. H. Blackman* that the growth of an annual plant follows a compound interest law. There are many natural phenomena in which the rate of change is proportional to the quantity itself. When money is placed at compound interest it accumulates in this way. As Blackman has pointed out, assimilation and growth are closely correlated: "If the rate of assimilation per unit area of leaf surface and the rate of respiration remain constant, and the size of the leaf system bears a constant relation to the dry-weight of the whole plant, then the rate of production of new material, as measured by dry-weight, will be proportional to the size of the plant, i.e. the plant in its increase in dry-weight will follow a compound interest law."

The ultimate dry-weight of an annual plant will depend in the first place upon the weight of the seed, and in the second place on the rate at which material already present is employed to produce new material, that is to say, the percentage increase in dry-weight per day or week or other period; and thirdly the time during which the plant increases in weight.

The final dry-weight is expressed by the formula:

$$W_1 = W_0 e^{rt}$$

where W_0 is the initial dry-weight and W_1 the final dry-weight, t the time, e the base of natural logarithms, 2·718, and r the average rate of interest. Blackman considers that r must be taken as an important physiological constant, because it represents the efficiency of the plant to add to its original capital, and he has termed it the "efficiency index" of the plant.

From a consideration of this suggestion of Blackman that the growth of an annual plant follows a compound interest law, two facts emerge: (1) that a large seed and (2) a high economy of working represented by a large efficiency index allow of the highest production of vegetative material by an annual plant. A good start will mean a larger capital to work on, for the efficiency of the plant is highest in its earliest stages of growth.

Robertson† has compared the S-shaped curve for growth to that of an autocatalytic reaction. The curve for an autocatalytic reaction is S-shaped. For example, the hydrolysis of an ester by water is an autocatalytic reaction. The reaction at first proceeds slowly, but increases in velocity with increase of acid formed by hydrolysis and then falls away.

* *Ann. Bot.*, 1919, **33**, 353; *New Phyt.*, 1920, **19**, 97.
† *Archiv. f. Entwickl. Org.*, 1908, **26**, 108.

Robertson has suggested from the shape of growth curves that growth is in the nature of a monomolecular autocatalytic reaction, and that there is some special catalyst that governs the growth rate of an organism. From experimental observations Robertson considered that in any particular growth-cycle, either of an organism as a whole or of any part of an organism, the greatest increase in weight or volume in any one particular unit of time occurs when the total growth due to the cycle is half accomplished. A growth-cycle of this nature conforms to the equation:

$$\log \frac{x}{A - x} = K(t - t_1)$$

where x is the amount of growth expressed either in terms of weight or of volume which has taken place in a time t, A is the total amount of growth attained in the course of the cycle, K is a constant, and t_1 the time in which half the growth cycle is completed.

If growth be governed by a catalyst and is of the nature of an autocatalytic reaction, once the maximum has been reached a constant value should be recorded. In practice a loss is found. This is considered to be due to secondary changes.

The whole question of the nature and interpretation of growth curves is still in a very controversial state, but the trend of recent investigations shows that many growth problems can be expressed by means of mathematical formula with a fair degree of accuracy. Thus Ashby has shown that the curves under given conditions for increase in frond-area, dry-weight and frond-number in the aquatic *Lemna minor* when plotted against time approximate to the exponential type, i.e. follow a compound interest law, and the numerical constants can be calculated. Since such figures can be quoted and considerable reliance can be placed upon them, it is clear that a greater insight is slowly being gained into the complex problems of growth.

ENVIRONMENTAL FACTORS AND GROWTH

Growth is a complex problem and environmental conditions subject the rate of growth of an organism to various modifications. The manner in which a plant reacts to external factors is extremely complicated and the facts are not easy to interpret. Some of the more important external factors and their influence on growth will now be considered.

TEMPERATURE

In normal plants growth and temperature are closely correlated. It has already been seen that for successful growth the rate of assimilation must be greater than the rate of respiration. Both these processes are increased by a rise in temperature, and it therefore follows, other things being equal, that an increase in temperature will bring about increase in growth. For normal plants the range of temperature in which growth is able to take place is restricted. Medium temperatures are the most favourable for growth, whereas very high or very low temperature brings growth to a standstill.

The effect of temperature on the growth rate of the roots of *Pisum sativum* has been investigated by Leitch,* who found for the temperature range −2° C. to 29° C. that a uniform curve of increase is obtained and resembles the curves for respiration and temperature obtained by Kuijper (see Chapter XIII). When the temperature was increased above 29° C. fluctuations began to make their appearance and no single curve expressed the relationship between growth and temperature. It was therefore necessary to express the rate of growth in successive periods of time by separate curves.

Minimum, maximum and optimum points of temperature for growth have been claimed by different investigators, but these values are really fictitious as has been pointed out before, because the Blackman time-factor comes into operation. For example, it was found by Leitch that at 30° C. and 35° C. the rate of growth of pea roots in the first 10 minutes is the highest attained, but in the first half-hour there is a rapid fall, followed by a second maximum showing recovery, after which there is a gradual fall, while at 40° C. there is no increase in growth and decrease is rapid and uniform. It is obvious that at 30° C. and 35° C., although there is rapid increase in the growth rate for a short period, the detrimental effect of these two temperatures comes into play with the lapse of time.

The variations in the growth rate discovered by Leitch at the higher temperature ranges may well be due to the operation of a number of different factors. It is possible that the activity of these different factors is increased by higher temperatures, but the rate of increase may not be uniform for each particular

* *Ann. Bot.*, 1916, **30**, 25.

factor concerned. Leitch's results show that between 10° C. and 20° C. the van't Hoff coefficient lies between 2 and 3, while Hicks has found that when *Lemna minor* is grown in a constant light intensity of 1,000 foot-candles that Q_{10} was 2·8 and that the optimum growth rate occurred at 30° C.

Plants exhibit a high degree of diversity in their aptitude for enduring wide ranges of temperature. Some plants, such as the alpine Soldanella, develop in the spring when they have to force their way through snow before the shoots reach the air. A number of bacteria are known which are able to multiply at 0° C., while others such as *Bacillus thermophilus* are able to multiply at 70° C., and cease to reproduce when the temperature falls to 42° C.

The investigations of Gregory* on the rate of increase in area of cucumber leaves when grown under conditions of continuous artificial light are of importance in this connection of the relationship between temperature and growth. Under these conditions he found that there was a continuous fall from the first measurement of area, and he claims that there is some "detrimental factor" involved which may have been the high temperatures which had to be maintained during the experiments. It is possible that these high temperatures may have increased the rate of respiration, and he has put forward three suggestions to account for the falling off of the growth rate:

(1) That growth stops through incipient starvation due to high respiration rates, the corollary of high temperatures.

(2) The detrimental influence of high temperatures and low light intensity may have been due to a change in the distribution of assimilated material in the plant.

(3) To a direct action on leaf growth or to the factors acting simultaneously.

Resistance of Plants to Low Temperatures.—The effect of intense cold on plants of economic importance is very variable. Some are able to survive this treatment, while others succumb. The question therefore arises as to the nature of the factors which allow some varieties to survive, whereas others are unable to withstand this treatment.

It has been assumed that the main cause of injury by freezing is the withdrawal of water from the cell and its freezing in the intercellular spaces. With further fall in the temperature, still more water is withdrawn by imbibition and becomes frozen on

* *Ann. Bot.*, 1921, **35**, 93; 1928, **42**, 469.

the cell walls. As more and more water is withdrawn from the cell, the force with which the remaining water in the cell is held increases rapidly, and there is a marked increase in the concentration of the cell sap. When, however, the plants are warmed slowly after freezing, the water that has become frozen in the intercellular spaces will be drawn into the cells once more and little injury will result. On the other hand, with prolonged freezing, permanent injury and death may result from such causes as desiccation of the protoplasm, precipitation of proteins in the cells, as a result of salting out owing to the high concentration of salts in the cells from withdrawal of water, and even from mechanical injury to the plasma-membrane.

The practice of hardening plants to low temperature by gradually subjecting them to lower and lower temperatures is one of considerable economic importance in horticulture, and a number of suggestions have been put forward to account for this acquired resistance by plants to very low temperatures. It was first suggested by McDougal that the pentosans played some important function in this connection. This view has been further extended by Hooker* who considered that the pentosans, or perhaps some specific pentosan, hold the water in the plant cells by adsorption, and that under these conditions the water does not become frozen. Further investigations by other workers, however, have not confirmed this view, and there is at the present time no entirely satisfactory theory to explain this process of hardening.

LIGHT (a)

The effect of light on the growth of a plant is varied and must be considered from several standpoints. In addition to the fact that the ordinary green plant requires light for photosynthesis, light has a formative effect upon plants. Thus the effect of light upon growth is both direct and indirect.

When plants are grown in complete darkness they exhibit a very different appearance from those grown in light, and the plants are said to be *etiolated.* Chlorophyll is not developed, in plants with internodes these become elongated, while rosette plants, such as *Bellis perennis*, form elongated stems in darkness with spirally arranged leaves. Moreover, the xylem development is poor, the leaf lamina is suppressed and becomes scale-like in

* *Univ. Miss. Agric. Exp. Stat. Bull.*, 1920, **40**, 120.

shape, and the etiolated shoot is soft, sappy and weak. In experiments on etiolation it is best to employ plants with abundant reserve supplies of food, such as the dahlia or potato.

Short exposures to light have a remarkable morphological effect upon etiolated plants. It was shown by Trumpf* that when *Phaseolus multiflorus* was exposed to short periods of artificial light daily, which varied from twelve hours to thirty minutes, that even the shortest exposure to light (30 minutes) tended to make the internodes shorter and the laminae of the leaves broader. When even shorter exposures to light were used (one minute to 30 minutes) marked morphological effects were produced. In plants exposed for 10 minutes, for example, well-developed laminae were produced, but no chlorophyll was formed.

This work has been fully confirmed by J. H. Priestley,† who also found that completely etiolated plants of *Vicia Faba* show no sign of lateral leaf development and always retain their plumular hook.

The anatomy of etoliated plants of *Vicia Faba, Solanum tuberosum* and *Pisum sativum* has been examined by Priestley and Ewing,‡ who found that under such conditions these plants developed a true primary endodermis with Casparian strip in the stem in place of a starch sheath. It has been suggested by Priestley§ that the presence of this primary endodermis restricts the supply of nutrient material necessary for growth to tissues within the endodermal cylinder and the shoot apex. As a result of the presence of this endodermis only the cells which cap the end of the endodermal cylinder receive adequate supplies of food, and growth in length of the stem is favoured at the expense of normal lateral growth of leaf and cortex.

Gregory,|| on the other hand, has suggested that the action of light may be of the nature of a "master" photochemical reaction, which is independent of photosynthesis, and as a result of this action of light some substance is formed which is directly responsible for leaf expansion. Thus, there is a certain minimum of light intensity needed for growth to proceed, and unless this intensity is maintained, a time-factor makes its appearance which leads to a continuous fall in relative leaf growth rate.

The effect of light of different wave-length on plant growth

* *Bot. Archiv.*, 1924, **5**, 381.
† *New Phyt.*, 1925, **24**, 271.
‡ Ibid., 1923, **22**, 30.
§ Ibid., 1926, **25**, 145.
|| *Ann. Bot.*, 1928, **42**, 469.

has been investigated from time to time, but only the more recent investigations are of any value. The older work on this subject paid no or little attention to the energy relations involved. In investigations of this kind it is necessary that the energy relations are the same in all the experiments. When plants, for example, are grown under, say, red and blue screens to discover the influence of red and blue rays on growth, the energy transmitted by the two types of screen must be the same.

According to Schanz,* who grew a number of different plants under glass which transmitted definite parts of the spectrum, taller plants are produced when rays of short wave-length are removed. Plants attained their maximum height under red light and showed a minimum under the blue-violet rays. The development of anthocyanin pigments apparently depends on ultra-violet light being present, for if light of short wave-length were removed the flowers were pale in colour. Schanz is of the general opinion that light of short wave-length is detrimental to growth.

It has been shown by Popp† for a wide variety of plants that the blue-violet rays are necessary for good and healthy growth. The absence of light of lower wave-length than $529\mu\mu$ results in more or less etiolation, and plants grown under such conditions uniformly flowered later, while some, such as sunflower, practically failed to flower at all. There was also a decrease in fresh and dry-weight, a deficiency in total carbohydrate and an increase in total nitrogen. It therefore seems clear that although the blue-violet part of the spectrum is necessary for normal, healthy development, ultra-violet light is not, for when these rays were excluded growth was normal.

The influence of the duration of light on growth is of great importance, and in the case of the appearance of the floral organs is covered by the term *photoperiodism*. An enormous mass of data has now been accumulated on this subject, which more properly belongs to a discussion on reproduction and will be further considered in Chapter XVI. Apart, however, from reproduction, duration of light has important results on vegetative growth.

Garner‡ and his co-workers, who have been pioneers in this matter, have shown that in such plants as tobacco, soy bean, etc., the amount of vegetative growth is proportional to the

* *Ber. deut. bot. Ges.*, 1919, **37**, 430.
† *Amer. J. Bot.*, 1926, **13**, 706.
‡ *J. Agric. Res.*, 1920, **18**, 553.

duration of daylight. When plants were exposed for short periods to daylight, they were found to have a slow growth rate and to be slender and small in habit. Not all plants, however, respond in this way. Adams* found that mustard, sunflower, tomato, flax and soy bean showed a greater average dry-weight, greater average height and earlier flowers when exposed to the longest day. These results are not in accord with Garner and Allard, for example, who found for soy bean that in plants illuminated daily for five hours flowering began in 27 days, whereas if the plants received a daily exposure of 12 hours, flowering did not take place for 110 days.

Another feature exhibited by plants exposed to different periods of illumination was the development of the rosette habit. It was found by Garner and Allard† that with sufficient departure from the optimum amount of light, growth of the primary axis could be suppressed, and aerial growth was now practically confined to leaf development; a type of development typified in rosette plants. The creeping or prostrate habit was also another manifestation of the sub-optimal action of light.

Tuberization is also said to be a feature of photoperiodism. It had been shown many years ago by Vöchting that tuberization in the potato is favoured by darkness, and he was able to obtain tubers on the primary shoot or on a branch by darkening these parts of the plant. It was found by Garner and Allard‡ that with short duration of daylight (5 hours) the onion showed no signs of forming bulbs, while potatoes failed to form tubers under increased illumination.

The effect of photoperiodism on root development has also been investigated. Garner and Allard ascertained that cuttings of the Biloxi soy bean made no top growth during the winter months, but when an examination was made of the root system in the spring, the soil was found to contain a mass of roots, which were quite out of proportion to the size of the plant judged by the standard of summer growth. According to Weaver and Himmel,§ who have investigated the effect of long and short periods of daily illumination on a large variety of plants, short day illumination resulted in a retardation of root and shoot system, and the greatest development of shoot and root occurred when a long daily illumination was used.

* *Ann. Bot.*, 1923, **37**, 75; 1924, **38**, 509.
† *J. Agric. Res.*, 1923, **23**, 871.
‡ Ibid., 1923, **23**, 871.
§ *Plant Physiol.*, 1929, **4**, 435.

Although suggestions have been put forward to account for why shorter or longer exposure of plants to illumination should bring about the various results that have been described above, we are still ignorant of the underlying causal mechanism. It will therefore serve no useful purpose to discuss this aspect of the matter here.

The effects of different quantities of light, which were measured by the product of the intensity of illumination (expressed as metre-candles) and the time of exposure to light (expressed in seconds), have been investigated by Blaauw* on certain plant organs.

Special precautions were taken in this work to have uniform material. As far as possible pure lines were used and due allowance was made for sampling and experimental errors. The plants were grown either in the dark or in red light under constant conditions of humidity and temperature. The experimental procedure was to illuminate the plants from above and by a special arrangement of mirrors equal illumination was ensured from all sides so that phototropic stimulation was avoided.

The plants to be examined after growing in the dark or in red light were exposed to a definite quantity of white light measured in metre-candle-seconds. After exposure to a definite intensity of light for a short period, the resulting growth in length of the plant organ in the dark was determined.

Blaauw found that the effect of illuminating the sporangiophores of the fungus *Phycomyces nitens* with quantities of light varying from 1 metre-candle-second to 4,000 metre-candle-seconds resulted in an acceleration of the growth rate, and this increase in growth was followed by a retardation and then by a series of accelerations and retardations until this light-growth response was over (Fig. 37). Thus it may be said that for each quantity of light that fell upon the sporangiophore of this plant there was an increase of growth. When similar experiments were performed upon the hypocotyl of sunflower seedlings with the same light-quantities (1 m.-c.-s. to 4,000 m.-c.-s.), a retardation of growth was discovered. With the roots of radish and oat no light-growth response was obtained; on the other hand, the roots of white mustard, *Brassica alba*, showed an increase of growth.

The light-growth responses of the coleoptile of the oat was

* *Zeit. f. Bot.*, 1914, **6**, 641; 1915, **7**, 465.

examined by Arisz,* who found that for quantities of light up to 4,000 metre-candle-seconds there was a retardation of growth. F. W. Went† employed 5,000 metre-candle-seconds and obtained a similar result. Arisz, however, obtained positive results with quantities of light greater than 4,000 metre-candle-seconds, and only obtained retardation once more when amounts greater than 70,000 metre-candle-seconds were employed.

Went discovered that the response of the oat coleoptile was in fact the resultant of at least two reactions, that of the tip and that of the base of the coleoptile. When the tip alone of the

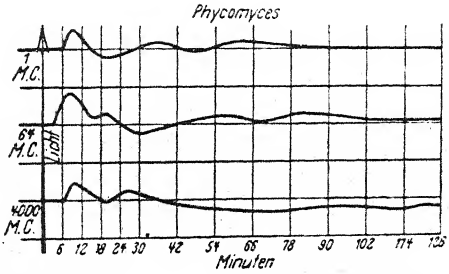


FIG. 37.—Light-growth reaction shown by sporangiophore of *Phycomyces nitens* to illuminations of 1, 64, and 4,000 metre-candle-seconds. The time at which the sporangiophore was illuminated is indicated by the arrow. (After Blaauw.)

coleoptile was illuminated, it was more sluggish in responding than the base, but although the reaction set in later, it was much stronger than that shown by the base. The result of illuminating the tip was a strong retardation of growth, which occurred from 20 to 30 minutes after exposure to light and was followed by a slight increase in growth, and the whole reaction was completed in 2 to 3 hours. When the base was illuminated it showed an earlier response than the tip of the coleoptile. Once more a retardation of growth took place, and this was followed by a slight acceleration and then the reaction came to a standstill (Fig. 38).

The light-growth response of the oat coleoptile has been explained by Went as follows: When the coleoptile tip is illuminated, the downward flow of auxin (for a description of this substance and its properties, see the section on Accessory Growth Factors) from the illuminated tip to the cells in the enlarging region of

* *Rec. Trans. bot. Néerl.*, 1915, **12**, 44.
† *K. Akad. van Wetenschappen. Amsterdam Proc. Sect. Sci.*, 1925, **29**, 185.

this organ is diminished. The evidence for this view was obtained by measuring the auxin-content of the tips of coleoptiles that had been grown in the dark and of others that had received 1,000 metre-candle-seconds illumination from above. The tips of the illuminated coleoptiles were then removed and placed eccentrically on decapitated stumps, when a 20 per cent less divergence from the vertical occurred than with tips which had not been illuminated in this way. Went therefore considered that 1,000

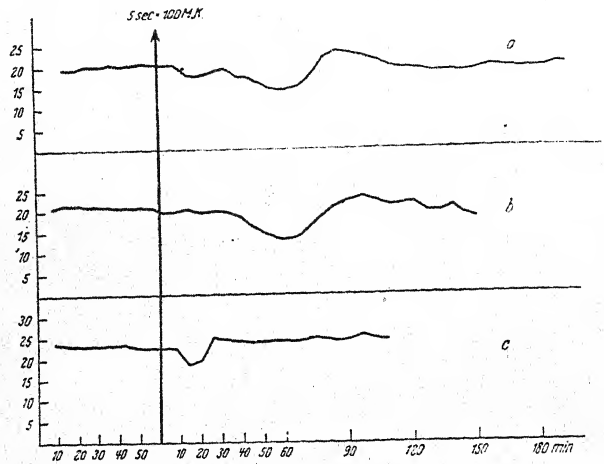


FIG. 38.—Light-growth response of oat coleoptile to a uniform illumination of 5,000 metre-candle-seconds. The arrow indicates time of exposure to light. A, Reaction of whole coleoptile when illuminated; B, Reaction when tip only is illuminated; C, Reaction when base alone illuminated. (After F. W. Went.)

metre-candle-seconds of light had either annulled or destroyed 20 per cent of the auxin.

Nevertheless, the destruction or inhibition of auxin by light is not a general phenomenon. It was found by van Overbeek* that the seedlings of Raphanus do not have a diminished auxin-content when illuminated, and the same amount of growth substance is transmitted through the hypocotyls in light as in darkness.

ACCESSORY GROWTH FACTORS

The problem of the raw materials of plant nutrition has already been discussed in Chapter XI. It is obvious that without the

* *Proc. Roy. Soc. Amsterdam*, 1932, **35**, 2.

proper variety and quantity of food the living plant cannot survive for any length of time. The scientific development of manuring of crops was largely elucidated in a long series of investigations by Lawes and Gilbert in their classical experiments carried out at Rothamsted.

Farmyard manure has been known for many generations to be an excellent all-round fertilizer for crops. Analysis of the manure has shown that it contains a large quantity of organic matter and a small proportion of ash—the ash being mainly composed of the elements potassium, sodium, magnesium, calcium, phosphorus and silica.

Three explanations have been put forward to account for the growth-promoting activity of farmyard manure. The first suggested that it was due to the presence of organic matter, the second to the ash-content, and the third, due to Lawes and Gilbert, to the ash-content plus the nitrogen of the organic matter.

The matter was experimentally tested by Lawes and Gilbert, who found that though the ash alone was ineffective, ash plus combined nitrogen in the form of ammonium sulphate acted as well as farmyard manure. This is made clear from the following table of values:

	Bushels per Acre Grain	*Cwt. per Acre Straw*
Farmyard manure	22	13
No manure	16	10
Ash of farmyard manure	16	10
Ash of farmyard manure + ammonium sulphate	$26\frac{1}{4}$	$15\frac{3}{4}$

Lawes and Gilbert's practical demonstration quickly led to the production and manufacture of artificial manures. They themselves saw the possibility of making up an effective artificial product by employing mixtures of mineral salts and nitrogen compounds. The great advantage of using such an artificial product is the fact that it is very much more concentrated in bulk than farmyard manure, so that there is a consequent saving in cartage.

Liebig had early announced the view that the effect of manures is proportional to the quantity used—a rule which Lawes and Gilbert showed to be modified by the law of diminishing returns.

The artificial manure used by Lawes and Gilbert was composed of potassium salts, phosphates and ammonium salts, the latter

to supply nitrogen. The effect of different quantities per acre of this mixture on wheat is shown in the table below:

Manure added per Acre in Pounds	1852–1894				1852–1912			
	Grain		Straw		Grain		Straw	
	Bushels per Acre	Increase	Cwts. per Acre	Increase	Bushels per Acre	Increase	Cwts. per Acre	Increase
None	18·3	—	16·6	—	14·5	—	12·1	—
43	28·6	10·3	27·1	10·5	23·2	8·7	21·4	9·3
86	37·1	8·5	38·1	11·0	32·1	8·9	32·9	11·5
129	39·0	1·9	42·7	4·6	36·6	4·5	41·1	8·2
172	39·5	0·5	46·6	3·9	—	—	—	—

A curious result has been found at Rothamsted by the prolonged use of artificial manure on the same ground. It has been discovered that the repeated use of artificials on the same plot leads to the gradual degradation of the crop, whereas plots fertilized with farmyard manure for over 80 years have shown no sign of deterioration. The cause of this strange result is at present unknown. There are several possibilities that may be considered. It is feasible that the natural manure in some way maintains the *status quo* of the colloidal nature of the soil, or it may assist the activity of the large micro-population of the soil. A further suggestion that has been made in this connection is the artificials lack some essential factor or factors which eventually leads to the deterioration of the crop.

It has already been seen in Chapter XI that a large number of plants need minute quantities of the element boron. *Vicia Faba*, for example, dies in a very characteristic manner if boron be absent, and that the nodules attached to the roots of this plant and which are responsible for the assimilation of molecular nitrogen, are much reduced in the absence of boron, and the bacteria contained in them change their symbiotic habit for one of parasitism and attack the protoplasm of the host plant. Besides such mineral elements as boron being necessary for normal healthy growth, other substances have been found that influence growth markedly such as the auximones and auxins or growth-regulators.

It was found by Bottomley* that when he grew the aquatic

* *Proc. Roy. Soc.* (*Lond.*), 1917, **89B**, 481; *Ann. Bot.*, 1920, **34**, 345, 353.

ferns Azolla and Salvinia, as well as the common duckweed *Lemna minor*, in ordinary mineral culture medium, such as Knop's or Detmer's solutions, healthy growth was not maintained and multiplication did not proceed normally. When, however, he added small amounts of organic matter, such as the aqueous extract of bacterized peat or material containing nucleic acids, normal healthy growth was obtained. He found that the amounts necessary to bring about this result were very small, and he suggested that some other factor is needed besides the ordinary mineral nutrients for the successful growth of these forms, and he gave the name *auximone* to this factor.

This work of Bottomley was contradicted in America, and it was pointed out that these aquatics will grow for months on end and multiply normally in a solution composed of purely inorganic salts, provided that the physiological balance of the solutions be correct. Bottomley's original work has, however, been fully confirmed by Ashby,* who has shown that the addition of small amounts of organic matter does have a very remarkable influence on the growth of Lemna.

In this work the Lemna plants were grown under carefully controlled conditions in dilute solutions of mineral salts alone, as well as with the addition of organic matter (aqueous extract of horse dung). Although the Lemna plants could be grown indefinitely in pure mineral solution, the addition of organic matter produced some remarkable results. The number of chloroplasts per frond, for example, were increased, and there was an increase in cell size as well as frond area in plants grown in culture solutions to which had been added 0·2 parts per million of an aqueous extract of horse dung. The influence of organic matter is apparently catalytic in nature, for an increase in amount added in greater concentration than 2·0 parts per million caused no increase in the growth rate.

GROWTH-REGULATORS OR AUXINS

A considerable amount of data has now been accumulated regarding the presence of substances in plants which are able to bring about cell enlargement. Actually, we owe the first steps of our knowledge of the presence of these substances to Boysen Jensen's work on phototropism, which will be further discussed in Chapter XVII. The great advances that have been made within

* *Ann. Bot.*, 1929, **43**, 805.

recent times in our knowledge of these products, both physiologically and chemically, has come from the work of the Dutch investigators at Utrecht, especially F. A. and F. W. Went, Kögl, Dolk, Heyn and others.

The coleoptile of Avena* has been mainly used for the physiological side of this work. There is apparently in the apex of this coleoptile a substance which diffuses down to the base of the organ and brings about enlargement of the cells. If the apex of the coleoptile (1 to 2 mm. of the tip) be removed, growth comes

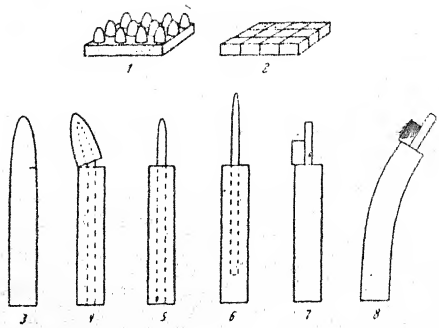


FIG. 39.—Technique of F. W. Went for extraction and experimental work with auxin. (1) Twelve coleoptile tips are placed on an agar plate; (2) The agar plate is next cut up into twelve sections; (3-8) Coleoptile decapitated and agar block placed eccentrically on stump. Note curvature of coleopile away from covered side. (After F. W. Went.)

to a standstill for a time. When, however, the tip is replaced on the decapitated stump by means of a thin layer of gelatin, growth commences again. Should the tip not be replaced upon the decapitated stump, growth will recommence after a time to a limited extent, owing to the formation of some fresh growth-promoting substance. If a second decapitation be performed two hours after the first, growth is permanently stopped, for any fresh growth-promoting substance that is formed in this period is removed.

Other facts concerned with this growth-promoting substance or substances, which are now known as *auxins*, are: If the base of a coleoptile be placed upon a decapitated stump, there is no increase in growth, or if the tip of a coleoptile be placed eccentrically upon a stump growth curvature takes place on the side away from the tip. This differential growth is said to be due

* See Went., *K. Akad. van Wetenschappen Amsterdam Proc. Sect. Sci.*, 1926, **30**, 10; *Rec. Trav. bot. Néerl.*, 1928, **25**, 1.

to the passage of a greater amount of auxin to the elongating cells on the side covered by the tip. Auxin can be extracted from the coleoptile tips by placing them on thin plates made of 3 per cent agar. That auxin actually diffuses out of the coleoptile tips is shown by the fact that if the tips be allowed to remain on the agar plate for one hour, which is then cut up into small blocks of equal size, and one of the blocks be placed eccentrically on a decapitated stump, curvature will take place on the side away from the one on which the block is placed. It was considered by Went, who elaborated this technique, that auxin had diffused out of the tip of the coleoptile into the agar, and had then diffused out of the agar into the stump of the coleoptile. In the stump the auxin had passed to the basal region and there brought about enlargement of the cells. If, instead of placing an agar block containing auxin eccentrically upon a stump, it is placed centrally, vertical growth of the stump takes place. It would therefore appear that in these circumstances the auxin has been uniformly distributed round the elongating coleoptile.

It is clear from these experimental results that the growth of an Avena coleoptile is governed or regulated by some definite chemical substance or substances. Such bodies are called *hormones*. The term hormone is defined as a chemical compound produced by one tissue which is able to exert or influence in some specific way the functional activity of another tissue. Hormone activity is well known in animal physiology and has been extensively studied. In plants, however, knowledge of their activity and chemical nature is still in the early stages of development.

Different species apparently all have the same auxin. The auxin from the coleoptile of Avena will stimulate the growth not only of the coleoptiles of other grasses, such as maize and barley, but also of other plants from unrelated families. The growth of a decapitated axis of *Bellis perennis* is stimulated by auxin from Avena coleoptile. The fungus *Rhizopus suinus* is an excellent source of auxin, and auxin isolated from the *R. suinus* promotes the growth of Avena coleoptile. The presence of auxin has been found in many dicotyledonous seedlings, such as *Lepidium sativum*, *Vicia Faba*, *Raphanus sativus* as well as in malt extract and in human and other mammalian urine. Human beings excrete between 1 to 2 mg. of auxin per day, irrespective of sex. It has been discovered that a diet of fat augments the auxin-content

of urine, and large quantities of this substance are excreted after a meal in which vegetable fats have been consumed.

Although auxin promotes the growth of coleoptiles, it has a different action upon the growth of roots. If the tips of maize roots be removed, it will be found that these organs still continue to elongate, and if the stumps be reheaded with the tips, growth is retarded. It has been shown by Cholodny* that if the root stumps be reheaded with coleoptile tips, growth is again retarded. Thus auxin brings about promotion of growth in the coleoptile, but retardation of growth in the root. Similarly, he found that root tips placed upon coleoptile stumps promoted growth. Thus, one and the same substance is able to bring about growth increase of one organ and growth retardation of another. Cholodny's work has been confirmed by Keeble, Nelson and Snow† and also by Hawker.‡ These investigators used F. W. Went's technique and placed agar blocks containing auxin from the root-tips of maize or of *Vicia Faba* on the decapitated roots of these plants. They showed that if a block of agar containing auxin were placed eccentrically on the stump, curvature occurred towards the side containing the agar block, whereas if the block were placed centrally upon the stump there was no curvature, presumably because growth had been retarded.

The Chemical Nature of Auxin.—There are a number of very considerable difficulties in the way of investigating the chemical nature of auxins. In the first place they are present in such minute amount in plant organs that this in itself is a heavy handicap, and it is safe to say that the brilliant advances that have been made on this side of the subject by Kögl and his co-workers would have been quite impossible without the use of micro-methods of analysis elaborated by Pregl.

It was found by Nielsen that the fungus *Rhizopus suinus* excretes into its culture medium a substance which markedly accelerates the growth of coleoptiles and retards the growth of roots. This substance is now called auxin A. Shortly afterwards the presence of such a body was demonstrated in yeast, as well as in the ascomycete, *Aspergillus niger*. It is possible that all these substances are identical in chemical nature, or at any rate very closely akin chemically. They were all found to have one property in common,

* *Ber. deut. bot. Ges.*, 1924, **42**, 356; *Jahrb. f. wiss. Bot.*, 1926, **65**, 447; *Planta*, 1928, **6**, 118; 1929, **7**, 461.

† *Proc. Roy. Soc.* (*Lond.*), 1929, **105**B, 493; 1931, **108**B, 537.

‡ *New Phyt.*, 1932, **31**, 321.

namely, solubility in ether. Nielsen was also able to show that *Rhizopus suinus* elaborates another product, which has been called auxin B, which possesses the power of increasing the dry-weight of *Aspergillus niger*. This compound, however, is insoluble in ether.

Kögl and Haagen-Smit* have isolated auxin A from a number of different sources, such as yeast, *Rhizopus suinus*, coleoptile of *Zea Mays* and human urine. The best source for the isolation and examination of the properties of auxin A was found to be human urine. From this source they were able to isolate a crystalline compound with a molecular weight between 330 and 353, and it was found to have the formula $C_{18}H_{32}O_5$. The term "Avena-Einheit" (Avena-unit = A.E.) has been introduced by Kögl and Haagen-Smit to compare the strengths of auxin isolated from different sources. A unit of A.E. is defined as being the amount of auxin contained in a block of 3 per cent agar of dimensions $2 \times 2 \times 0{\cdot}5$ mm., which brings about a curvature of 10° in two hours at a temperature of 22 to 23° C. when placed eccentrically on one decapitated stump of Avena coleoptile. They ascertained that the crystalline product they had obtained from human urine had an efficiency of 30,000,000 A.E. In other words, 1 mg. of this substance could impart a curvature of 10° to 30,000,000 Avena coleoptiles.

The isolation of auxin A from human urine is a complicated process, and is based on the fact that this substance is acidic in nature and forms sparingly soluble lead salts. The bicarbonate-soluble fraction of urine was submitted to prolonged extraction with light petroleum, and the residue remaining after the light petroleum had been distilled off was treated with lead acetate in weakly alkaline 70 per cent alcohol. The lead salt obtained in this way was then converted into the calcium salt, and the latter heated with acid methyl alcohol. Instead of a methyl ester being obtained, a lactone was formed, and this was obtained in a crystalline condition by distillation *in vacuo*. The product finally isolated by Kögl and Haagen-Smit was found to be a monobasic acid (auxin A), with the formula $C_{18}H_{32}O_5$ and melting-point 196° C. The formula of the lactone proved to be $C_{18}H_{30}O_4$ and its melting-point was 173° C. Both acid and lactone were found to be physiologically active.

The formation of a tridinitrobenzoyl derivative showed the

* *Proc. Akad. Wetenschaffer Amsterdam*, 1931, **34**, 1411; *Z. physiol. Chem.*, 1933, **214**, 241; 1933, **216**, 31; 1933, **220**, 137, 162; *Z. angew. Chem.*, 1933, **46**, 469; *Z. physiol. Chem.*, 1934, **225**, 215; **227**, 51; **228**, 90, 104, 113.

presence of three hydroxyl groups in the molecule. Auxin A itself is feebly laevorotatory and shows mutarotation, owing to lactone formation, equilibrium being reached in 1 to 2 hours. On these grounds, Kögl and Haagen-Smit inferred that auxin A must be a δ-hydroxy-acid and not a γ-hydroxy acid, since acids of the latter type take a much longer period to reach equilibrium. The physiological activities of both auxin A and its lactone disappear on keeping, due possibly to the formation of a pseudo-auxin. A single double bond was found to be present in the molecule, and on catalytic reduction an inactive dihydro-compound was obtained with the formula $C_{18}H_{34}O_5$. Since this compound is fully saturated, auxin A must contain a carbon ring in the molecule, for a straight-chain compound would have the formula $C_{18}H_{36}O_5$.

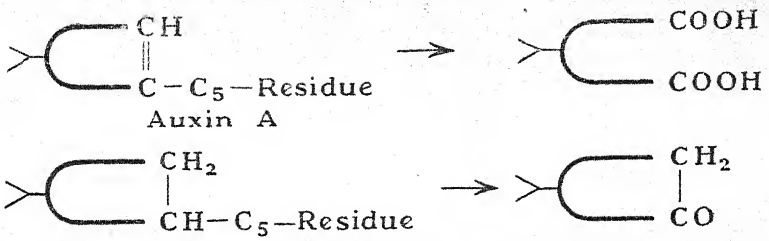
When auxin A was oxidized with alkaline permanganate, a dibasic acid, $C_{13}H_{24}O_4$, was isolated, presumably owing to the rupture of the molecule at the double bond. This new acid was found to be devoid of hydroxyl groups. It was not a malonic acid and contained no keto-group. The assumption was made that the original carboxyl group in auxin A had been eliminated on oxidation, together with a portion of the C_5-residue, which must in this case carry all three hydroxyl groupings, and the two carboxyl groups that were found to be present in the new derivative must have been formed by the opening of the unsaturated ring. The alternative view that one of the two carboxyl groups in the molecule of the new acid is that present in auxin A itself is not tenable, for the hydroxyl group in the δ-position to it must be removed in the course of this oxidation, thus:

$$\mathrm{R\,.\,C(CO\,.\,OH) = CH\,.\,\overset{|}{\underset{|}{C}}\,.\,(OH) \rightarrow R\,.\,CO\,.\,CO\,.\,OH}$$

or

$$\mathrm{R\,.\,CH(CO\,.\,OH)\,.\,CH = \overset{|}{\underset{|}{C}}\,.\,C(OH) \rightarrow R\,.\,CH(CO\,.\,OH)_2}$$

Further confirmation that the two carboxyl groups are introduced by the opening of an unsaturated ring is given by the oxidation of dihydro-auxin with chromic acid, when a ketone, $C_{13}H_{24}O$, is produced. Once more the C_5-residue has been eliminated, leaving a carbonyl grouping, but the ring, being saturated, has not been opened. This can be shown as follows:



Auxin A

Dihydroauxin

The C_{13} acid, on heating, readily forms an anhydride and not a cyclic ketone, and is therefore a glutaric acid. Hence it may be inferred that the unsaturated ring in the molecule of auxin A is five-membered. Furthermore, the C_5-residue must be attached to one of the unsaturated carbon atoms, and the other must carry a hydrogen atom to account for the formation of a dibasic acid on oxidation.

In addition to auxin A, Kögl and his co-workers have been able to isolate from maize germ and malt another closely related compound, auxin B, which also acts as a growth regulator. Auxin B has been found to be present in human urine, the fungus *Boletus edulis*, *Rhizopus suinus*, as well as in blood and milk. Examination of auxin B from vegetable sources shows that it is necessary to have another factor present (Co-B), which is deficient in a number of these products. Zinc salts as well as filter paper appear to fulfil the functions of a complementary body.

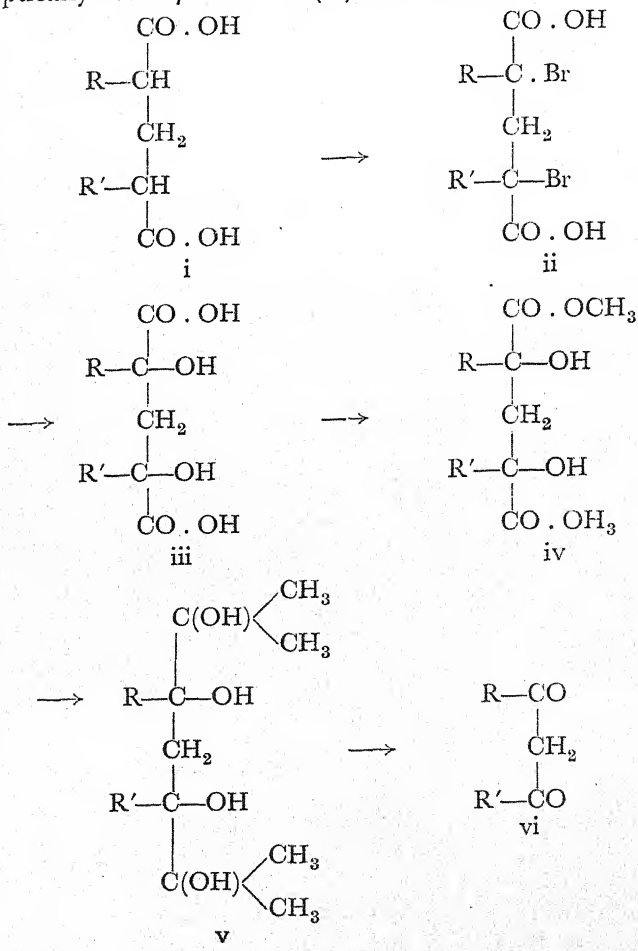
Auxin B is without influence on the coleoptile of Avena, but accelerates the growth of *Aspergillus niger*. It has a marked effect in increasing mycelium production, whereas auxin A does not affect the dry-weight of mycelium produced or the numbers of conidia, but does bring about a slightly accelerated formation of conidia and subsequent degeneration.

The formula of auxin B was found to be $C_{18}H_{30}O_4$, and it is isomeric with the lactone of auxin A. It contains only one hydroxyl group in the molecule and a carbonyl group is also present. When heated it loses a molecule of carbon dioxide, and gives rise to a neutral ketone. It is therefore a β-ketonic acid. On oxidation with alkaline permanganate, it gives rise to the same dicarboxylic acid as auxin A. Thus the remainder of the molecule must have the same structure as auxin A.

It is clear that the final elucidation of the structure of auxin A and of auxin B depends upon the elucidation of the structure of

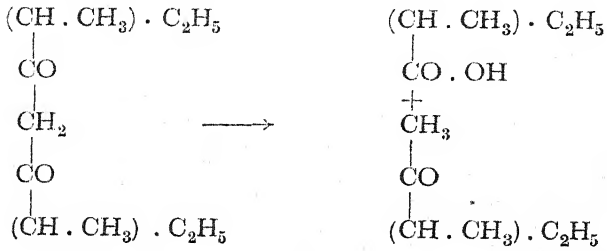
the optically active acid $C_{13}H_{24}O_4$, which it has already been suggested is a glutaric acid. Kögl and his co-workers have now been able to achieve this task, although the total amount of material available was only 90 mg.

The acid (i) was first brominated, when a dibromo-derivative was obtained, and this was then treated with silver oxide (ii). The silver salt of the dihydroxy-acid thus formed (iii) was allowed to react with methyl iodide, when the dimethyl ester (iv) was obtained. The dimethyl ester obtained in this way was submitted to the Grignard reaction, using methyl-magnesium iodide (v). The glycol so formed was treated with lead tetracetate, when an optically active β-diketone (vi) was isolated:

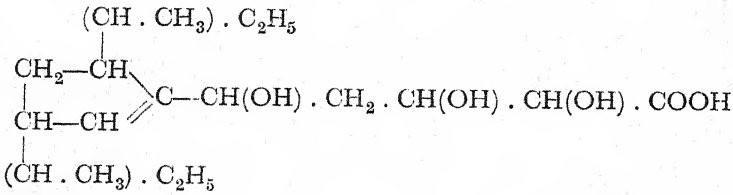


Now such an acid could not have been formed from an acid of the succinic series, since this would lead to the formation of an α-diketone, nor could it have been formed from a $\beta\beta'$-disubstituted glutaric acid, for this would have given rise to a dialkylated (and therefore not enolizable) malondialdehyde.

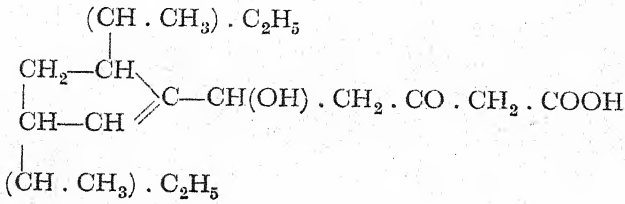
The diketone isolated from these reactions, when submitted to alkaline hydrolysis, gave rise to *d*-α-methylbutyric acid and methyl *sec.*-butyl ketone. The methyl *sec.*-butyl ketone proved to be optically inactive, probably due to racemization. These products being homogeneous, the parent ketone itself must have had a symmetrical structure:



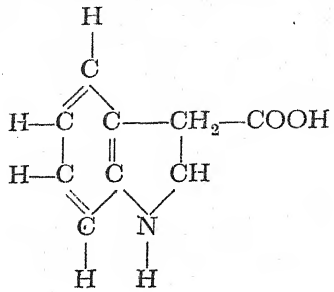
The C_{13} acid is therefore $\alpha\alpha'$-di-*sec.*-butylglutaric acid and the constitution of auxin A may be written:



and that of auxin B as:



A third physiologically active substance has now been isolated from human urine by Kögl, which has proved to be indole-3-acetic acid, $C_{10}H_9O_2N$. This has been called hetero-auxin, and has the constitution:



Hetero-auxin is produced by the bacterial decomposition of tryptophane, and according to Kögl it may occur in appreciable amounts in urine under conditions which have led to the bacterial population of the intestine reaching abnormal numbers.

A number of other substances have now been announced as being physiologically active, such as 1-methylindole-3-acetic acid (ethyl ester inactive), 2-methylindole-3-acetic acid (methyl ester inactive), indole-3-pyruvic acid and a number of other substances, but up to the present no clear relationship has been discovered between constitution and physiological properties.

Translocation of Auxin.—F. W. Went in his physiological experiments with auxin attempted to determine the mode of transport of this substance down the coleoptile of Avena. He discovered that the cells of the coleoptile show protoplasmic streaming and considered this phenomenon to be responsible for the downward translocation of auxin.

Van der Weij* has used auxin prepared from urine on a number of observations of the translocation of this compound down the coleoptile of Avena. Agar blocks containing known concentrations of auxin were placed on the decapitated tops of coleoptiles, which were allowed to rest on a block of pure agar. The influence of temperature, initial concentration of auxin, length of coleoptile and orientation of coleoptile on the translocation of auxin was then determined. The "velocity" of transport was measured by the time taken for the first appreciable trace of auxin to reach the lower block of agar, and the "intensity" of transport was determined by the amount of auxin that subsequently reached the lower block of agar in unit time. He ascertained that the "intensity" of transport through paths of different length were almost equal, and the "velocity" of

* *Rec. Trav. bot. Néerl.*, 1932, **29**, 381.

transport was nearly independent of temperature. He also demonstrated that the "intensity" of transport increases with increase in the initial concentration, whereas the "velocity" is apparently unaltered. Van der Weij does not agree with Went that protoplasmic streaming is a factor in the transport of auxin, since the "velocity" of transport is nearly independent of temperature, and protoplasmic streaming is markedly affected by temperature and has a high temperature coefficient.

The Mode of Action of Auxin.—F. W. Went has put forward the suggestion that the mode of action of auxin is to modify the process of extension in cell size. It is evident that the method of action of auxin must be to increase cell extension and not cell division, for the cells in the lower region of the coleoptile which react to the influence of auxin have ceased to divide. It was considered by Went that auxin in some way makes the cell walls more plastic so that they suffer irreversible stretching beyond their limit of elasticity by the turgor pressure of the cell sap, and in this manner the length of the cells is permanently increased.

Whatever may be the exact mechanism whereby auxin is able to bring about cell extension, it appears to be complex in the extreme. According to Thimann and Bonner,* auxin does not act directly upon the cell wall by the formation of new material, nor does it modify the permeability of the wall, but it appears to act in some indirect way through the protoplasm and the action is catalytic in nature. This is rather borne out by the observations of Bonner,† who found that cell elongation will only take place in the presence of oxygen, and that it is suppressed by the presence of hydrogen cyanide in concentrations which suppress the rate of respiration of the tissues. As is to be expected, the concentration of auxin decreases from the tip to the base of the coleoptile of Avena, and when plant tissues are crushed, the growth hormone is inactivated, possibly owing to the activity of the oxidase system. If, however, the tissues be crushed under chloroform, auxin can be recovered, and this method of isolation allows of its assay in tissues.

Bonner and Thimann‡ were able to show that auxin disappears continuously during growth, and that the amount of growth produced is proportional to the amount of hormone that has

* *Proc. Roy. Soc.* (*Lond.*), 1933, **113B**, 126.
† *Protoplasma*, 1934, **21**, 406; *Proc. Nat. Acad. Sci.*, 1934, **20**, 393.
‡ *J. Gen. Physiol.*, 1935, **18**, 649.

disappeared. The reason for the disappearance of the hormone, or how it disappears, is not known. Its disappearance apparently has nothing to do with the respiratory processes of the cell, since the presence of hydrogen cyanide does not affect the rate of disappearance.

It is to be observed that it is only the free acid and not any salt of auxin A that is physiologically active. It was early found by Dolk and Thimann* that if the hormone were applied by means of agar blocks which had been buffered to a pH = 7·0, no curvature was produced in an Avena coleoptile. According to Strugger,† the growth of sunflower seedlings, *Helianthus annuus*, is markedly stimulated by their immersion in acid buffer solutions, and if a strip of epidermis be removed in decapitated hypocotyls (and presumably on this account auxin-free) permanent curvature away from the wound is produced. Strugger has suggested that the method of action of auxin in promoting cell elongation in the coleoptile of Avena is to regulate the course of cellular metabolism in such a way as to produce acid conditions, and in this way to influence the rate of growth, which is related to the difference between the pH and iso-electric point of the protoplasm of the cell.

Bonner has also found that coleoptile sections grow much faster when infiltrated with dilute acid, and that this growth acceleration is accompanied by an increase in the plasticity of the cell wall and is approximately proportional to the concentration of free acid which would be produced from auxin already present in the cell in the form of salts. Bonner regards the stimulative action of acids on decapitated coleoptiles as being due to the conversion of the physiologically inactive salts of auxin remaining in the stump into the physiologically active acid.

It has already been seen that auxin promotes the growth of coleoptiles, hypocotyls of seedlings, stalks and other aerial parts of plants, and retards the growth of roots. The precise reason why one and the same substance should bring about elongation of the cells in one set of tissues and retardation of growth in another still remains to be determined.

CORRELATION

Although it is the frequent practice in physiological investigations on the higher plants to use individual organs for a par-

* *Proc. Nat. Acad. Sci.*, 1931, **18**, 30. † *Ber. deut. bot. Ges.*, 1932, **50**, 77.

ticular purpose, it must be kept in mind that the different parts of a plant are interrelated. For example, investigations on photosynthesis are often carried out on detached leaves, or investigations of the permeability of cells on isolated pieces of tissue, and the tacit assumption is made that these isolated tissues or portions of tissues are behaving as complete entities. In many physiological investigations it is impossible to deal with whole plants, and it is necessary to resort to the use of individual organs, so that the cheap gibe is frequently made that a plant physiological investigation is concerned with the erection of elaborate apparatus, and the material to be investigated is a cherry laurel leaf left under a bell jar.

Nevertheless, it is clear that the different organs of a plant are interrelated in their physiological activities. The roots absorb water from the soil as well as dissolved salts, and these are transported to the leaves and used for various metabolic purposes. Carbon dioxide is absorbed from the air by the leaves and in the presence of light and chlorophyll converted into carbohydrates, and these are in turn used for respiration and translocated to other parts of the plant for storage and converted into new tissue in growth. On this view the activity of the whole plant may be represented by the simple summation of the activities of the different parts.

The problem of correlation is, however, more complex than has been suggested above. The activity of the organism as a whole may differ in many respects from a mere summation of the properties of each of its individual organs. Functions may be suppressed or modified by the association of different organs within the whole plant. If the terminal bud of a seedling of monopodial growth be removed, the axillary buds grow out and form new shoots. It is evident that the apex exerts some influence which prevents the development of axillary shoots. Again, when a cambium cell divides, one of the daughter cells is destined to become a xylem element and the other a phloem cell, and the question arises as to why mere difference in position should cause this alteration in the nature of the tissues.

As early as 1882 it was suggested by Sachs that root-formation in cuttings of stem and root might be due to the activity of special root-forming substances. In other words, the stimulus involved here is chemical in nature. It was also considered by Jost that definite chemical substances might be involved. This view has

since been brilliantly verified by the discovery of auxins and other examples of hormone activity in plants.

The inhibitory effect of an apical bud on the development of lateral shoots has been very fully investigated by Snow* on *Vicia Faba*, *Phaseolus multiflorus* and *Pisum sativum*.

In the first place it was shown by Snow that in *Vicia Faba*, as well as *Phaseolus multiflorus*, when the epicotyl was ringed so that all tissues including the cambium were removed external to the wood, there was no axillary growth, so that the inhibitory power of the terminal bud is still able to exert its influence in a stem that has been ringed. Control plants were used which were decapitated but not ringed, and these showed signs of regeneration within four days. Even when the main apex and axillary bud were connected by the xylem with a few cells of pith parenchyma adhering to it, the inhibition was still able to pass down although it was considerably weakened in its effects. In another experiment, the stem of *Vicia Faba* was killed for a short distance by allowing a jet of steam to play upon it for 20 or 30 seconds. In this case the axillary bud grew out below the treated zone, and apparently the inhibition was unable to pass down owing to the "physiological shock" that this treatment had induced, and the main stem continued its growth. It was further shown by Snow that the inhibition is able to pass a watery gap.

According to Snow the inhibitory substance formed at the apex is transported through the living tissues, and, in stems in which a zone has been killed, leaks into the xylem just below the dead zone. Having leaked into the xylem, it is then drawn up in the transpiration stream across the zone of dead tissue and leaks out once more into the living cells, and in this way prevents the development of the axillary bud.

Snow has attempted to show exactly what part of the apex of the shoot brings about inhibition of the axillaries. It is known that in certain plants the larger leaves are also able to bring about inhibition. The plant mainly used in this investigation was *Pisum sativum*. Here the leaves are arranged in two ranks (distichous), and it was discovered that axillaries of the first leaves could be made to develop by the removal of all the leaves from the stem from below upwards to those about 2·5 mm. in length. If continuous growth of the axillaries is to be maintained,

* *Ann. Bot.*, 1925, **39**, 841; 1929, **43**, 261; *New Phyt.*, 1929, **28**, 345; *Proc. Roy. Soc. (Lond.)*, 1931, **108B**, 209, 305; 1932, **111B**, 86.

then the largest leaves of the terminal bud must be removed as they develop. If this were not done, the growth of the axillaries was stopped. Thus a considerable amount of the inhibitory effect of the intact shoot must be derived from leaves that have reached a certain size.

The inhibitory power of a leaf was found to increase with the age of a seedling, and consequently with the height of the leaf on the plant. In small seedlings 20 mm. in height the inhibitory effect is very weak. Moreover, a single leaf is unable to inhibit completely, so that in a normal shoot inhibition must be due to action of several leaves acting in concert. It was also discovered by Snow that the partial inhibitory power of a leaf gradually increases with age to a maximum value and then falls away, until a full-size leaf shows practically no inhibitory influence. Almost the entire inhibitory effect of the shoot in *Pisum sativum* comes from three of its leaves, i.e. those between 2 to 6 mm., 6 to 18 mm., and 18 to 30 mm. in length respectively in each growth interval. Of these three leaves, two still form part of the terminal bud, and the third is only a small way beneath it.

The strength of the inhibition increases with the length of the intervening stem in *P. sativum*. If young pea seedlings be decapitated in the epicotyl, two shoots will be developed from the axils of the cotyledons. The question as to why the two shoots developed in this way when nearly of equal length do not inhibit the development of one another has been examined by Snow. When the shoots are not of equal length the weaker shoot stops growing and after some weeks dies. Presumably in this case the stronger shoot has inhibited the growth of the weaker, for if the more strongly growing shoot be removed, the weaker one develops indefinitely. In seedlings in which the two shoots were nearly of equal length, if one were defoliated from below upwards until only the small leaves at the apex remained, the defoliated shoot grew very much less than the intact shoot, and in the end growth ceased altogether and the shoot eventually died. On the other hand, if one shoot were defoliated in the manner described above, and the other intact shoot were removed, growth of the defoliated shoot continued. Thus defoliation does not arrest the growth of a pea shoot, provided that it is the only one of a seedling. It has been suggested by Snow that when two intact and equal cotyledonary axillaries are present on the same plant, they are in a state of equilibrium. Should this state of equilibrium be upset in

any way, such as defoliation of one of the shoots, the balance is destroyed, and it is rapidly inhibited from further development by the intact shoot and ultimately killed.

It is evident from this work of Snow's that there is good reason to suppose that inhibition of bud development and stem elongation is due to hormone activity.

The problem of the inhibition of bud development has been examined by Thimann and Skoog* in *Vicia Faba* and other plants. Terminal buds were removed and placed on agar, and the usual technique devised by Went was then carried out. The agar was cut into small blocks and applied to decapitated coleoptiles of Avena. It was found that the terminal buds of *V. Faba* produce auxin in rather large quantities, but the amount falls off with age of the plant. To show that auxin is the true inhibitor of lateral bud development, it is necessary that the following conditions be satisfied: (1) The growth substance is produced by the terminal bud; (2) the lateral buds, once they begin to develop, also produce auxin in appreciable amount; (3) auxin is produced in leaves, and (4) the application of auxin, once the terminal bud has been removed, inhibits the development of the lateral buds to the same degree as does the terminal bud of intact plants.

It was shown by Thimann and Skoog that these various conditions could be satisfied. It was found by them that when plants of *V. Faba* which had been grown in the light were treated with growth substance obtained from *Rhizopus suinus,* auxin B, and hetero-auxin, inhibition of lateral bud development occurred. Difficulties were encountered when auxin A prepared from urine was used. This substance loses its physiological properties on keeping, and owing to delays it was found that the inhibition brought about by the use of auxin A was small, but nevertheless significant. The most complete inhibition was produced with heteroauxin. This result may possibly be due to the fact that both auxin A and auxin B show a decrease in their power of activity on keeping, whereas heteroauxin remains constant and therefore retains its full activity throughout the experimental period. Moreover, heteroauxin is much less susceptible to oxidizing agents than either auxin A or B and will on this account be less inactivated in the plant.

Healthy young plants of *Vicia Faba* of various ages were decapitated 1 to 2 mm. below the terminal bud. Each bud was

* *Proc. Roy. Soc.* (*Lond.*), 1934, **114B**, 317; *Proc. Nat. Acad. Sci.*, 1934, **20**, 480.

then placed on a block of 1·5 per cent agar for 4 hours. The blocks were then divided into 12 pieces and tested upon Avena coleoptiles. In all cases curvature occurred, the degree of curvature depending on the age of the plant; the older the plant the less growth substance was formed in the terminal bud. Thus a bud from a plant 12·1 cm. in height produced a curvature of 11·7° in Avena coleoptile, whereas one 37·3 cm. in height produced no curvature at all. It was also found that whereas undeveloped lateral buds show little or no production of growth substance, when they become larger there is an appreciable amount formed, about one-half that of the young terminal bud. Furthermore, it was ascertained that leaves also produce growth substance, but in lesser amount than in the bud. The amount produced in a leaf is approximately inversely proportional to the age of the leaf.

Experiments were made to see whether inhibition was produced when agar blocks, which had previously been allowed to stand, would bring about inhibition of lateral bud development if applied to a decapitated stem. The blocks of agar were renewed every 6 hours, for this was found to be the approximate time that it took for the utilization of the growth substance. Inhibition as complete as that in the intact plant was obtained.

The production of growth substance in *V. Faba* was found to occur only in the light, but the response of the stem to applied growth substance was greater in the dark than in the light, i.e. there was greater elongation of the stem in darkness through the application of growth substance.

It is clear from this work that the auxin of Avena and the inhibitor of bud development in *V. Faba* are the same. It is an odd fact, and one for which there is at present no satisfactory explanation, that one and the same substance promotes stem elongation but retards lateral bud development. In this connection compare the activity of auxin A on coleoptiles and roots (see above).

It was shown in 1893 by Jost that, as a general rule, although there are exceptions, growth in stems only takes place under the influence of leaves that are still in an active state of growth, and further, that the influence exerted by these leaves, whatever may be its nature, travels in the downward direction. The suggestion was made in 1924 by Karstens* that this "cambial stimulus," as

* *Mitt. Inst. Allg. Bot., Hamburg*, 1924, **33**, 6.

it has been termed, is of hormone nature. This problem has been reinvestigated by Snow,* who has been able to demonstrate that the cambial stimulus is able to pass across a protoplasmic discontinuity, such as a piece of moist linen. He also repeated some experiments of Jost in which stems were partially split in the longitudinal direction, so that a portion of the cut stem was attached to the parent shoot either at the upper or lower end. With *V. Faba* it was found that whereas the cut portion which was attached at the upper end increased in diameter and also grew in length, the strips of tissue attached at the lower end did not grow. Snow considers that these results support the view that there is a downward flow of a substance from the apical region of the shoot which promotes the elongation of the short internodes.

It will be recalled that over fifty years ago it was suggested by Sachs that root-formation in cuttings of stem and root might be due to the activities of special root-forming substances. There is now available good evidence that this is really the case. It was found by F. W. Went† that when the detached leaves of Acalypha and *Carica papaya* were placed with their petioles resting in water, and this water was later evaporated at a low temperature to a small bulk, a root-promoting substance had apparently diffused out of the petioles into the water. This water was mixed with warm 3 per cent agar and the whole allowed to set. When this agar was applied to portions near the top of defoliated stem cuttings of Acalypha, the development of adventitious roots at the base was promoted. A similar result was obtained when a preparation of diastase was used, but boiling of the diastase did not destroy its root-promoting activities, so that this result could not have been due to the enzyme itself, as this is thermo-labile, but must have been due to some other product present in the diastase preparation.

Some further investigations by Thimann and Went‡ show that this root-promoting substance or hormone has a wide distribution in the plant world. The name rhizocaline has been given to this substance. It was found to be present in rice polishing that had been extracted with water, wheat germ extracted with potassium bicarbonate, the pollen of many plants, such as *Acer*

* *New Phyt.*, 1933, **32**, 288.
† *Zesde Internat. Bot. Congres Amsterdam Proc.*, 1935, **11**, 267.
‡ *K. Akad. van Wetenschappen Amsterdam. Proc. Sect. Sci.*, 1934, **37**, 3.

Negundo, *Hicoria cordiformis*, *Quercus alba*, Juniperus and *Sequoia sempervirens*. It was also found to be present in the leaves of *Prunus Laurocerasus*, *Helianthus annuus* and of Malva, and in the etiolated buds and shoots of *Pisum sativum*. Its presence was also detected in urine excreted at different times of the day.

From such chemical tests as have been performed, it would appear that rhizocaline is an unsaturated organic acid of about the same acid strength and solubilities as auxin A. Like auxin A it forms a lactone. It is susceptible to oxidation by such reagents as potassium permanganate, hydrogen peroxide, but not benzoyl peroxide. There is thus no doubt that it is an unsaturated compound, but it is doubtful if it is identical with auxin A, although the two hormones are very similar in their chemical properties. Wide discrepancies, for example, were found in the ratio of root units to growth substance units in a given preparation, which is evidence against complete identity.

CHAPTER XVI

REPRODUCTION

THE birth of a new individual from a parent organism is called reproduction. The term *individual* has a definite significance in zoology, and connotes the process whereby a fresh organism is produced by the union of a male gamete (sperm) with a female gamete (ovum), i.e. the formation of a new individual by sexual reproduction. It is not, however, an easy matter to define satisfactorily the term *individual* as far as plants are concerned. It is a commonplace of horticultural practice to produce a number of independent plants by means of cuttings. These are independent individuals, but the term reproduction is not applied to this method of plant multiplication. The term *vegetative multiplication* or *propagation* is usually used in this connection. In vegetative multiplication the new plant does not arise afresh from the beginning.

Reproduction in plants takes place in two ways, (1) by the formation of spores, *asexual* reproduction, and (2) by the union of a male gamete with a female gamete, *sexual* reproduction. Both asexual and sexual reproduction occurs in most plants, and in the higher land plants both types of reproduction take place at definite points in the life cycle.

The production of reproductive bodies by plants may be looked upon as a phase of development. In certain cases, reproduction is a very definite phase. Thus in annual plants, which complete their life-cycle in a single season, reproduction may be regarded as the final phase of development. The formation of flowers, fertilization and the ripening of the fruit are all successive steps immediately prior to the death of the plant. In other species there may be vegetative development for a long number of years before the life of the plant is terminated at the completion of the reproductive phase. An example of this condition is to be found in *Agave americana*, in which vegetative development may continue for as long as 70 years or even more when flowers and fruit are produced and the death of the plant occurs. In some cases reproductive organs are formed before vegetative development takes place. The Magnolia produces flowers early in the spring, and it is only later that vegetative development is renewed for the new growing season.

It has been known for a long time that the factors which govern vegetative development are antagonistic to those concerned with reproduction. It was considered at one period that special internal factors must govern the formation and development of the reproductive organs. The classical experiments of Klebs, however, demonstrated that the phase of reproduction is governed by certain definite external conditions, and that reproduction does not take place from internal causes alone, although these also play an important part. It was further shown by Klebs that while conditions for active vegetative development are present, reproduction does not occur. Finally, he was able to demonstrate that the factors which control reproduction are more limited than those concerned in vegetative development, so that reproduction is likely to be inhibited by too high or too low intensity of some factor.

The larger part of Klebs' investigations was concerned with the lower plants, such as algae and fungi. The results that have been obtained using higher plants for physiological experiments on reproduction have never been quite satisfactory or convincing, mainly owing to the difficulty of devising suitable experiments.

It was discovered by Klebs that under favourable conditions, the green alga, Ulothrix, which inhabits freshwater streams merely grows vegetatively. Should, however, the supply of oxygen be limited or the flow of water stopped, zoospores are formed. Klebs' early work on Ulothrix was not particularly critical, and he obtained more remarkable results with two species of Oedogonium, *Oe. capillare* and *Oe. diplandrum*. In *Oe. diplandrum* darkening had a marked effect on asexual reproduction, which was increased by the addition of a 4 per cent solution of cane sugar. After having cultivated this plant in running water in which it assimilated vigorously, it formed swarm spores on being transferred to a dilute nutrient solution. Thus asexual reproduction in *Oe. diplandrum* sets in when it is removed from running water to still water, by the lowering of the temperature and the presence of a nutrient medium. Sexual reproduction in either of these species takes place when the water is limited in amount and if it contains small supplies of nutrient salts and the plants are strongly illuminated.

Klebs also obtained some very striking results with fungi. Many fungi will remain in the vegetative condition almost in-

definitely, and in a number of cases it is difficult to persuade them to form reproductive organs. The phycomycete Saprolegnia, for example, can be grown for many years without the appearance of zoosporangia or sexual organs. Klebs found that when a well-nourished mycelium of *Saprolegnia mixta* was placed in distilled water it formed sporangia. If it were transferred to a 0·1 per cent solution of haemoglobin or leucin, there was at first active vegetative growth, and then the sexual organs, oogonia and antheridia, made their appearance.

These few examples from Klebs' extensive investigations will suffice to show that reproductive and vegetative development are dependent on different circumstances and that active vegetative growth is inimical to active reproduction. This problem of the relation of growth to reproduction has been studied by Coons* for the fungus *Plenodomus fuscomaculans*, which is an active parasite of the apple.

When the fungus was grown on conductivity water there was only a small amount of growth, when distilled water was used as a medium, better growth was obtained and a few pycnidia were formed. Thus under these experimental conditions conductivity water was the lower limit of vegetative growth and distilled water for reproduction. As the food supply was increased there was increase in reproduction up to a certain limit. Many media which were found to be suitable at the start proved to be unfavourable at the end. If the medium were at all acidic reproduction was inhibited. When Coons employed a synthetic medium containing varying amounts of potassium dihydrogen phosphate (M/100), magnesium sulphate (M/300), maltose (M/100) and asparagine (M/500), he found that increase or decrease of maltose or asparagine had marked effects upon reproduction. He also ascertained that light was a necessary factor for reproduction, as well as abundant aeration. He was able to replace the stimulus of light by the addition of a few drops of hydrogen peroxide, nitric acid, potassium permanganate or ferric chloride. It will be observed that all these substances are powerful oxidizing agents.

The suggestion has been made by Coons, that among the different parts of an organism there is strong competition for oxygen, and that under conditions which favour vegetative growth the available oxygen is used for ordinary metabolic

* *J. Agric. Res.*, 1916, 5, 713.

processes. When, however, the food supply becomes reduced, a so-called "hunger-state" sets in and ordinary respiration is lowered. Should the organism when it is in this condition be stimulated by light or some oxidizing agent, oxidation of the richer cell contents will take place, such as the fats and proteins, and a large amount of energy will be released. "This energy is used for reshaping the reserve foodstuffs into complex protein bodies, the spores."

Other examples of whether the vegetative or reproductive phase takes place through the influence of external factors are also to be obtained among the fungi. Many fungi will not fruit in the light, while others will not fruit in the dark. The ascomycete *Pyronema confluens*, which is frequently to be found growing on ground that has recently been burnt, is a case in point. It has been shown by W. Robinson* that a sequence of causation can be recognized in this form. The initial stage in the sequence is the definite arrest of development of growth in the main hyphae of the mycelium, followed by development of the lateral branch systems, which in artificial culture grow into the air owing to spacing conditions on the agar surface. In these circumstances moisture relations become altered, and the effect of the energy of light is shown in morphological changes following upon the development of antheridia and oogonia. The aerial branches have the potentiality of developing into the reproductive structures before they have received energy from light. This is shown by the formation of abortive structures which arise when the cultures are grown in darkness in equivalent positions to apothecia in normal cultures grown in the light. Light operates relatively late upon the regions in the mycelium when the potentiality for development has already been determined. The absorption of a certain amount of energy from light is thus a final phase in the sequence of causation concerned in development. Moisture relations have also to be suitable for reproduction. The most favourable conditions for reproduction were found to be between relative humidities of 50 and 70 per cent. Below a relative humidity of 15 per cent or near 100 per cent, no antheridia, oogonia or apothecia are formed. Thus, in this form both light and humidity play an important part in reproduction and act by setting in motion a train of events already prepared by the internal condition of the mycelium.

* *Ann. Bot.*, 1926, **40**, 245.

The effect of different external factors on the reproduction of the higher plants can now be considered.

VERNALIZATION

The term "Vernalization" is the latinized form of the Russian word, "Jarovizacija," which means the process of pre-sowing treatment. Vernalization is an agricultural method of accelerating the development of plants. Through vernalization, it is possible to make winter plants bear fruit in the first year, and to convert late flowering forms into early ones. It is obvious that a treatment that is able to bring about such results must have very great economic implications.

Although differing in details, the technique of vernalization is essentially very simple. The seed is first soaked, so that the dormant condition is broken. This preliminary soaking, however, is not allowed to proceed to completion, so that growth is not allowed to go forward at full speed. It is possible to retard the growth of such partially soaked seed for a long time at the initial stages of germination, when the tip of the young root has just emerged through the seed coat. By retarding the growth of seeds at this stage it is possible to subject them to the influence of the necessary factors which will eventually bring about the desired acceleration of later development.

Very full practical details for vernalization of different plants have been given by Whyte and Hudson,* and in this connection the monograph issued by the Imperial Bureau of Plant Genetics *Vernalization and Phasic Development of Plants* should also be consulted. Two examples will suffice here. In winter wheat of normal moisture-content (12 to 14 per cent) the amount of water required for the preliminary soaking process is 37 litres per 100 kg. of seed. Late spring varieties require less water (33 litres) and early spring varieties even less (31 litres). Unless these full amounts of water are used vernalization will not proceed normally. The water must be applied in three successive stages so as to ensure complete absorption, and thorough mixing is also necessary for the same reason. Winter wheat must be kept in the pile for 2 to 3 days, the temperature being adjusted to 5 to 10° C. Under these conditions preliminary germination will begin and when 3 to 5 per cent of the grain have burst their coats, the temperature

* *Imp. Bur. Pt. Gen.*, 1933, Bull. 9.

is reduced to the value necessary for each variety (−2° C. in the case of winter wheat). The vernalization period is counted from this time.

Varietal idiosyncrasies are shown with regard to temperature requirements during the vernalization period. Thus winter cereals must not be treated at a higher temperature than 2 to 3° C., and not below 0° C., late spring varieties require a slightly higher temperature during vernalization, not below 3° C. and not above 5 to 6° C., whereas the early spring varieties of both hard and soft wheat need a still higher temperature, 8 to 10° C., but not higher than 15° C.

The longest period of vernalization is required by the winter cereals, depending of course upon the variety used. Thus a variety of wheat known as Kroperatorka requires a vernalization period of 40 days, the variety Ukrainka 45 days and Hostianum 0237, 50 days. The spring cereals require a much lessened period of vernalization, usually from 11 to 15 days, and the early spring varieties of both hard and soft wheats as short a period as 5 to 6 days.

In a second class of plants, which includes millet, soy bean and cotton, high and not low temperatures must be employed during the time of vernalization. In this class of so-called "thermophilic" plants, seed is partially soaked as before and the germinating seed is then exposed for several days to a temperature of 20 to 25° C. In some varieties even higher temperatures are required, up to 25 to 30° C. By this preliminary treatment these varieties are able to pass rapidly through the various stages of development up to fruit-bearing in cold climates, whereas if unvernalized plants had been used development would have been much retarded or even completely stopped.

An important practical point about vernalization is that the seed should not be immediately sown after treatment. It may be dried and sown later, but the preliminary treatment still remains effective.

Although a number of sporadic investigations have been made from time to time on the effect of temperature conditions upon the early stages of the development of a plant on the time of formation of the reproductive organs, we owe a great deal of our present knowledge of vernalization to the very thorough and extensive investigations of the Russian worker, Lysenko. The theoretical aspect of vernalization was also considered by

Lysenko, who suggested: (i) Growth and development are not identical phenomena; (ii) the entire process of the development of an annual seed plant consists of individual steps or stages; (iii) the stages always proceed in a strict sequence and a subsequent stage cannot set in until the preceding stage has been completed; (iv) different stages of development of the same plant require for their completion different external conditions.

Growth is defined by Lysenko as an increase in weight and volume of the plant at any particular stage. It is an unfortunate fact that the term vernalization has come to have a twofold meaning. It is not only used to describe the method of pretreatment of seeds so as to accelerate development, but the term has also been used for the first developmental stage which winter cereals are incapable of completing naturally when sown in the spring. It is better to use the word vernalization for the method of treatment of the seed, and to employ the term "thermo-stage" for the first developmental stage. A full consideration of Lysenko's theoretical views of vernalization would take us beyond the limits of a text of this nature. A critical analysis of his suggestions has been made by Maximov,* which should be consulted in this connection. The subject is in far too controversial a stage at the present time for any discussion to be profitable. It should also be mentioned that Lysenko is not the only author who has ventured to make theoretical deductions regarding the course of events involved in vernalization. There are a number of others who have all expressed themselves upon this question from time to time, and the more important of these various views will be found in the monograph published by the Imperial Bureau of Plant Genetics which has been mentioned above.

PHOTOPERIODISM AND THE CARBOHYDRATE/NITROGEN RATIO

The effect of duration of light on vegetative development has already been briefly discussed (see Chapter XV). The influence of duration of light on reproduction must now be considered. The literature on this aspect of the subject is enormous and can only be briefly described.

We owe the first systematic investigations on this question of light duration and plant response to Garner and Allard.† It was

* *Imp. Bur. Pl. Gen.*, 1934, Bull. **16**.
† *J. Agric. Res.*, 1920, **18**, 553; 1923, **23**, 871.

found by Garner and Allard that in such plants as the soy bean and tobacco that not only does light have an important influence upon the vegetative development of the plants, but that it is an extremely important factor in initiating the inception of the reproductive phase. They found that the majority of species investigated by them could be placed in one of two groups with regard to the response they showed in reproductive activity to different periods of illumination. To one group they gave the name "short-day" plants, for in this group the flowering stage was accelerated by a relatively short daily exposure to light, whereas long exposure to light daily either inhibited or considerably delayed the reproductive phase. The second group was termed by them "long-day" plants. In this group, which contains a fewer number of plants than the "short-day" division, prolonged duration of daily exposure to light led to the acceleration of the reproductive phase, whereas a short exposure retarded it. The term *photoperiodism* was coined by Garner and Allard to express this relationship between time of flowering and the daily length of the period of illumination.

Reduction of the light period below the optimum for stem elongation appears to lead to flower formation, while decrease of the optimum for flower formation leads in some cases to intense tuberization. In one experiment on *Cosmos sulphineus*, Garner and Allard* grew the plants in special ventilated light-proof boxes, in which three sides were removable, so that different portions of the primary stem of the plant could be exposed to daily periods of light and in certain cases to periods of darkness. It was found that when the upper portion of the stem was exposed to the full length of a summer's day and the lower portion to ten hours of light only per day, the latter soon flowered, while the former remained vegetative. In the same way, when the central part of the axis was exposed to the full length of a summer's day, while the upper and lower portions received only short exposures to light daily, the central portion remained vegetative, while the upper and lower portions of the stem flowered promptly.

Garner and Allard's investigations have been repeated in Canada by Adams.† This author is not in entire agreement with their results and found that soy bean, for example, under Canadian conditions behaves as a "long-day" plant, for he obtained the earliest flowers when the plants were exposed to the longest daily

* *J. Agric. Res.*, 1925, **31**, 555. † *Ann. Bot.*, 1923, **37**, 75; 1924, **38**, 509.

periods of light. On the other hand, Tincker,* working in Wales, has been able to confirm fully Garner and Allard's investigations. He was able to show that the runner bean (*Phaseolus multiflorus*), soy bean and Chrysanthemum (var. Mrs. William Buckingham) are all "short-day" plants. The onset of the flowering period was accelerated by short-day exposure. Certain grasses, such as *Anthoxanthum odoratum*, *Alopecurus pratensis*, *Dactylis glomerata*, *Lolium perenne* and *Avena sativa* proved to be "long-day" plants, and the onset of flowering period was retarded by short daily exposures to light.

Temperature and the humidity of the atmosphere must also be taken into account in certain cases of the response of plants to relative day length. Gilbert† has carried out some investigations on the connection between these three factors and considers that in *Xanthium pennsylvanicum* the relations between temperature and relative day length are as follows: Short-day plants (plants exposed to daylight for 10 hours each) with high temperatures give indications of flowering from 12 to 15 days after planting. When the daily exposure to light is increased (13 to 14 hours) and a high temperature is maintained, flower production takes place 47 days after planting. With low temperatures, plants exposed daily to 10 hours of light continued in the vegetative condition for 116 days before staminate buds were produced, whereas if the daily exposure were increased to 13 to 14 hours, the vegetative period was reduced to 92 days.

The question must be discussed here as to the nature of the fundamental causal relations between duration of exposure to light and the response of the plant. It has been claimed that the relationship known as the carbohydrate/nitrogen (C/N) ratio explains this response, and this ratio has been dragged in on every possible occasion as an "explanation" of why plants respond in the various ways that they do to duration of daily periods of illumination.

This carbohydrate-nitrogen ratio was first introduced by Kraus and Kraybill‡ in an elaborate biochemical investigation concerned with the nitrate and carbohydrate-content of the tomato and the responses correlated with their presence. Kraus and Kraybill were able to recognize four main conditions:

* *Ann. Bot.*, 1925, **39**, 721; 1928, **42**, 101; *J. Roy. Hort. Soc.*, 1929, **54**, 354.
† Ibid., 1926, **40**, 315; *Bot. Gaz.*, 1926, **81**, 1.
‡ *Oregon Agric. Coll. Exp. Stat. Bull.*, 1918, Bull. **149**.

(I) Though there be present an abundance of moisture and mineral nutrients including nitrates, yet without available carbohydrate supply, vegetative growth is weakened and the plants are non-fruitful.

(II) An abundance of moisture and mineral nutrients, especially nitrates, coupled with available carbohydrate supply, makes for increased vegetation, barrenness and sterility.

(III) A relative decrease of nitrates in proportion to the carbohydrate makes for the accumulation of the latter and also for fruitfulness, fertility and lessened vegetation.

(IV) A further reduction of nitrates without inhibiting a possible increase in carbohydrates, makes for a suppression both of vegetation and fruitfulness.

According to Kraus and Kraybill, "fruitfulness is associated neither with the highest nitrates nor with the highest carbohydrates, but with a condition of balance between them."

A large number of investigations have been published as a result of Kraus and Kraybill's original formulation of this carbohydrate/nitrogen ratio. It was thought that investigations based on this ratio might lead to an understanding of the physiological processes underlying such empirical horticultural processes as manuring and pruning. It is very doubtful if the C/N ratio has really helped very much in explaining these various phenomena. For a time the ratio was used by investigators in a very catholic way to explain nearly every manifestation of the plant, and went far beyond the boundaries set by the originators of the ratio. As Work* has pointed out: "When two factors play as complex and various parts in plant metabolism as do nitrogen and carbohydrates, each being present in more than one form and each being assigned a multiple rôle, it is hardly to be expected that their relation to each other may be expressed by a simple mathematical ratio. This would imply that the two are interdependent variables, and that together they constitute a factor which conditions the activity of the plant—in this case, vegetative and reproductive activity."

The C/N ratio was found by Gurjur† to vary between 2 and 19 for the tomato but fruiting only occurred when the ratio lay between the narrow limits of 4 and 6. According to Woo,‡ the ratio may be different for different plants to produce the same

* *Cornell Univ. Agric. Exp. Stat. Mem.*, 1924, Mem. **75**.
† *Science*, 1920, **51**, 351. ‡ *Bot. Gaz.*, 1919, **68**, 313.

range of effects. In *Ameranthus retroflexus,* which has apparently an abnormal capacity for the absorption and retention of nitrates, large amounts of nitrates may be present and yet the plant may not be forced out of reproduction, although the C/N ratio is low.

One of the main advocates in this country of the view that the C/N ratio is of importance in explaining the various results of photoperiodism is Tincker. From his chemical investigations which were carried out on plants which had been submitted to this treatment of photoperiodism, he has found that there is considerable correlation between chemical composition as expressed by this ratio and the behaviour of the plant to varying periods of daily illumination.

According to Tincker, the length of day influences stem elongation and controls the utilization of the products of photosynthesis. By this means the carbohydrate/nitrogen ratio of the tissues is influenced. "In general there would appear to be a correlation between the carbohydrate/nitrogen ratio and the behaviour of the plant. This does not necessarily signify that the magnitude of the ratio determines the behaviour of the plant and the nature of the growth made—the reverse may equally well be the true interpretation of the facts."

The general employment of the carbohydrate/nitrogen ratio in connection with photoperiodism raises one material difficulty. A distinction has to be maintained between "long-day" and "short-day" plants, and the application of the C/N ratio to explain the behaviour of "long-day" plants is not on the face of things unreasonable, since it may be supposed that the abnormal light period increases the amount of carbohydrate manufactured in photosynthesis. In this way it may be supposed that a correct C/N ratio is established at an earlier stage than would otherwise be possible. When, however, the behaviour of "short-day" plants is considered, difficulties quickly become apparent. In "short-day" plants short periods of daily illumination accelerate flower formation, and it is difficult to see how this result can be fitted into any scheme based on the C/N ratio. A short daily exposure to light must presumably give rise to a lessened amount of photosynthate formed by the plant.

There is an alternative hypothesis to explain photoperiodism which is more satisfactory than the one based entirely on the carbohydrate/nitrogen ratio. Since some plants are prevented

from flowering by exposure to increasing periods of daylight, quite irrespective of the light intensity, it seems evident that the synthesis of carbohydrate is not involved. On the other hand, it has been found that the amount of nitrate assimilated by the plant to more reduced nitrogen compounds is greater in light than in darkness, and is independent of the intensity of the light. The rate of reduction of nitrate in plant tissues is closely paralleled by the amount of the enzyme reductase that is present. From these data the suggestion has been put forward that the length of day affects the production of reductase in the tissues and this in its turn influences the amount of elaborated nitrogen in the plant, and so the balance between carbohydrate and nitrogen.

The investigations of Nightingale* and Eckerson† have shown that "short-day" plants exposed to short-day periods of daylight possess adequate assimilated nitrogen, excess of carbohydrate, and reductase is present and flowers are produced. When, however, short-day plants are exposed to long-day conditions of daylight they are high in nitrogen, low in carbohydrate (which is used in combining with the nitrogen), very high in reductase, and they are highly vegetative and non-fruitful. "Long-day" plants exposed to short-day conditions are poorly developed, low in nitrogen and very low in reductase and non-fruitful, but when submitted to long-day conditions of daylight, reductase and adequate nitrogen is present as well as excess carbohydrate, and flowers are formed.

If this scheme is true for all flowering plants, the chief difference between long- and short-day plants is to be found in their rates of reductase formation under different exposures to light. Under short-day conditions "long-day" plants produce reductase too slowly, whereas "short-day" plants produce it too rapidly under long-day conditions. It should therefore be possible to convert a "short-day" plant into a "long-day" one by starving it of nitrogen, and this has been done. It ought also to be possible to convert a "long-day" plant into a "short-day" one by raising the temperature and thus increasing the rate of respiration, so that excess carbohydrate is removed. The claim has been made that this has been accomplished.

* *Wisconsin Agric. Exp. Stat.*, 1927, Bull. **74**.
† *Cont. Boyce Thompson Inst.*, 1932, **4**, 119.

SEX

In the vast majority of Angiosperms, the male and female organs, stamens and ovaries, are borne on the same structure, the flower. In some species, however, the sexes are separated, e.g. Melandrium, Humulus, Rumex and Fragaria. Thus for the vast majority of the Angiosperms the question of sex differentiation does not arise, but the case is quite otherwise for the unisexual forms.

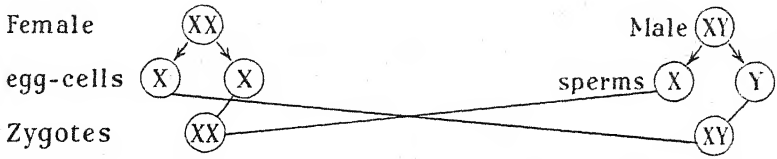
The question arises here: Is there a mechanism in the cell which brings about sex differentiation in unisexual plants? In normal sexual reproduction a male cell fuses with a female cell, and from this fusion product, the zygote, there arises a new individual. In animals the development of the zygote gives rise to either a male or a female individual.

It was observed over 30 years ago that in certain insects an anomalous body which looks like a chromosome makes its appearance during meiosis, but which behaves rather differently from the remaining chromosomes. It lags behind the chromosomes in the first meiotic division and in the second division passes undivided to one pole of the cell. It was originally suggested by McClung that this body is a true chromosome and has to do with sex-determination. This suggestion has since been amply verified. It is now known that in many species of animals the female has two such similar chromosomes, XX, and the male two unlike chromosomes, XY, or the male may have only one such chromosome, i.e. XO. Thus whether an individual is to be a male or female is determined at fertilization, and will depend upon whether the egg nucleus fuses with a sperm nucleus containing an X or a Y chromosome. In the case of animals in which there is no Y chromosome in the male, a female will result when an egg nucleus unites with a sperm nucleus containing X, whereas a male will result if the sperm has no X chromosome.

In most bisexual animals the male is heterozygous for sex, XY or XO, and the female is homozygous, XX. In birds, a few fishes and the Lepidoptera, the female is heterozygous for sex, ZW, and the male homozygous, ZZ.

It has been shown that the X chromosome like the other chromosomes of the nucleus carries certain heritable characters. The Y chromosome appears to carry no characters and seems to act rather like the dummy at bridge. The fact that the X chromosome carries heritable characters leads to certain peculiarities in

inheritance. It will be seen from the following diagram that the X chromosome is transmitted in a rather curious way. The male always transmits his X chromosome to his daughters, but never to his sons, while the sons receive their X chromosome from their mother:



This gives sex-linked inheritance, as it is called, a character of its own. A large number of cases of sex-linked inheritance are now known in animals, including man. The tortoise-shell cat is one such case, colour blindness in man another, and a large number of examples have been found in the fruit-fly *Drosophila melanogaster* by Morgan and his co-workers.

Sex chromosomes have now been described in certain unisexual plants. In Elodea, Humulus, and Rumex the female appears to be XX and the male XY. In the Bryophyte Sphaerocarpus the XX and XY mechanism also appears to be present.

So far we have seen that the presence of two X chromosomes determines whether an individual is to be a female, whereas if an X and Y chromosome are present in the zygote, the individual will develop into a male. The question may be asked why a mere alteration in the number of X chromosomes should bring about an alteration in the sex of the individual. Some experiments on Drosophila by Bridges, one of Morgan's co-workers, are of importance in this connection.

It was found by Bridges that in a triploid female of Drosophila, i.e., a form with 3 chromosomes of each kind in place of the normal 2, that when this female was used for breeding, some of its progeny were intermediate in their sex. These intermediates varied in character, some were more female in type, and others more male, but they were quite easily distinguishable from the normal males and females.

When a cytological examination was made of these intermediates, it was found that it was possible to establish a relation between the sex chromosomes and the appearance of the fly. The important factor in the determination of the sex of the various types was not the absolute number of X chromosomes

present, but the relation between the number of X and the number of each of the other chromosomes, the autosomes. If we call a single set of autosomes A, then the chromosomal composition of an ordinary female would be 2A + 2X, whereas an ordinary male would be 2A + 1X. The chromosomal composition of the triploid female was found to be 3A + 3X. This fly, with the exception that its eyes were larger than those of an ordinary female and possessed certain other small differences, did not differ from an ordinary female of composition 2A + 2X. It was found, however, by Bridges, that on breeding from this triploid, he obtained individuals of the composition 3A + 2X. These forms were intersexes. Two other types were also obtained, 2A + 3X, and 3A + 1X, the former were called "super-females" and the latter "super-males." On the whole they resembled the ordinary females and males, but were distinct types and always sterile.

It is clear from these facts that a factor or chromosome does not act independently but in co-operation with the other factors or chromosomes of the living organism. They also show that by altering the relative proportions of the factors or chromosomes, but without actually changing the nature of these constituents, the appearance of an individual may be altered.

Thus far we have seen from the existence of these intersexes, that sex need not be a sharply defined character, although in animals such appears to be the case at first sight. The intersexes described above have resulted from a change in the chromosomal composition of the cell, but intersexes can be brought about as a result of changes in the environment.

In certain animals the external environment has a very considerable effect on development. In the marine worm Bonellia, whether the fertilized egg develops into a male or a female depends upon whether the egg falls free, when a female is developed, or whether it falls upon the proboscis of the female when a male is formed. Presumably in this particular case the genetical conditions are so evenly balanced that sex is determined solely by external conditions. Another example of this state of affairs is given by the mollusc *Crepidula plana*, in which if the eggs be kept away from full-grown individuals they develop into females, whereas if they be allowed to develop near older specimens they become males. Frogs furnish still another example. It is possible in frogs for a zygote with two X chromosomes, which would

normally develop into a female, to be converted into a male by alteration in the environment.

Sex reversal has also been brought about in monoecious plants by changes in external conditions. It was found by Schaffner* that the sexual expression of *Zea Mays* is definitely affected by the length of daily illumination. Under correct conditions of moisture, nutrient supply and temperature, the amount of reversal was found to be directly proportional to the period of daily illumination. Intensity of illumination was found to be the decisive factor of sex determination. When maize was planted in the greenhouse on November 1st, with correct heat and suitable substratum 100 per cent of individuals were obtained with some degree of female expression in the tassel (male inflorescence), whereas if the maize were planted in the spring or summer, only pure staminate tassels were obtained. If the corn were planted after this date, under similar external conditions, sex reversal was found to be inversely proportional to the length of daily illumination. With the onset of equal periods of day and night little or no reversal was found to occur in the tassel. Schaffner† was also able to bring about sex reversal in the hemp. When this plant was exposed to short periods of daily illumination a considerable amount of sex reversal took place; about 90 per cent of male and female individuals. When the daily period of illumination was increased no reversal of sex occurred.

HETEROTHALLISM

Among the fungi sexual reproduction takes place by the union of two uninucleate or multinucleate cells which may be similar in structure or differentiated into antheridium and oogonium. The problem of sex in the fungi affords some interesting peculiarities.

It was found by Blakeslee‡ that the formation of zygospores in the Mucorales only takes place in certain members when hyphae of two different strains come into contact. In the absence of the opposite strain asexual reproduction continues indefinitely. Blakeslee termed these two strains "minus" and "plus." Though morphologically similar they are physiologically different, and

* *Bot. Gaz.*, 1927, **84**, 440; 1930, **90**, 279.
† *Ecology*, 1923, **4**, 323.
‡ *Proc. Amer. Acad. Arts and Sci.*, 1904, **40**, 205.

it is only when the two strains come into contact that zygospores are produced. Certain species of the Mucorales, however, are known in which zygospore formation takes place without two distinct strains being necessary. *Sporodinia grandis* is an example of such a form. Blakeslee termed those forms in which two distinct strains were necessary for zygospore formation "heterothallic" in contradistinction to the so-called "homothallic" forms in which this physiological specialization does not exist.

Until Blakeslee's discovery, a number of suggestions had been put forward to account for the very erratic appearance of zygospores in certain Mucorales. Blakeslee* and his co-workers have attempted to distinguish between the two strains by certain biochemical tests. For example, they used the "Gosio Reaction" for this purpose. The "Gosio Reaction" involves the use of selenium and tellurium salts. Various salts of these elements are reduced to their respective elements by the living cell. Blakeslee found that sodium or potassium tellurite gave the best results, rather than the corresponding potassium or sodium salt of selenium. Another test that was also used was Manilov's reagent (reduction of potassium permanganate). It was found, using either the "Gosio Reaction" or Manilov's test, that on the average, the (+) strains of Mucor (sp.), *Phycomyces nitens*, *Rhizopus nigricans*, *Absidia Blakesleana* and *Parasitella simplex* showed a greater power of reduction than the (−) strains. In respect to their reaction towards either of these reagents (+) strains of Mucorales behave like the female sex of the higher animals and dioecious green plants, and the (−) like the male sex.

The case of *Parasitella simplex* is very interesting in this connection of sexuality in the Mucorales. This is a very unusual form, and unlike the rest of the Mucorales which are saprophytes, *P. simplex* is a parasite on other members of the Mucorales. It was first suggested by Burgeff† that the parasitism of this species had developed by way of an imperfect sexual reaction. Burgeff, for example, was able to show that with species of Rhizopus, both the plus and minus strains of Parasitella formed galls with either strain of the host, whereas when Absidia was the host plant, Parasitella only formed galls when the opposite strains in the two cases were used. Thus the (+) strain of Parasitella only formed galls with the (−) strain of *Absidia glauca* or *A. caerulea*, and *vice*

* *Science*, 1920, **51**, 375, 403; *Bot. Gaz.*, 1921, **71**, 75; **72**, 162.
† *Botan. Abnendt.*, 1924, **4**, 1.

versa. Satina and Blakeslee* found that Burgeff's description of the curious cytological phenomena which accompany gall formation was perfectly correct. The cell contents of host and parasite mingle with the simultaneous formation of the gall. Later, outgrowths arise from the base of the gall and are subtended by a thick-walled storage cell, and they were able to show that there is communication between gall and storage cell.

Blakeslee has been able to show that the heterothallic Mucors are all strictly dimorphic, and there is no evidence of sex intergrades. It has been suggested by him that the reason for this absence of sex intergrades in the heterothallic forms is due to the fact that in the Mucors we are dealing with gametophytes, whereas in dioecious green plants, in which such intergrades are known, we are dealing with sporophytes with the diploid number of chromosomes.

Heterothallism has now been discovered in other members of the Phycomycetes, as well as in the Ascomycetes and Basidiomycetes. The situation presented by the Basidiomycetes is very complex. It was shown by Bensaude† in France and independently by Kniep‡ in Germany, that the Hymenomycetes show the phenomenon of heterothallism. Bensaude, for example, using *Coprinus fimitarius*, obtained four mycelia, each of which was from single spore culture. Of these four mycelia, only two survived. When they were subcultured over a period of eight months, they remained in the so-called "primary" condition, i.e. they developed no clamp-connections, nor did they show paired nuclei, and remained sterile. When cultures from these two mycelia were mixed, hyphal fusions occurred, and a "secondary" mycelium was formed in which the nuclei were found to be paired and divided conjugately, the division of each dikaryon being accompanied by wall formation with a clamp-connection, and fruit bodies made their appearance. Similar results to Bensaude were obtained by Kniep for the species *Schizophyllum commune*, but he found that fruit formation did not necessarily depend upon the presence of paired nuclei in the mycelium. He was able to show that a haploid mycelium derived from a single spore culture produced fruit bodies which in appearance were quite normal

* *Proc. Nat. Acad. Sci.*, 1925, **11**, 528; 1926, **12**, 191, 202; *Bot. Gaz.*, 1930, **90**, 299.

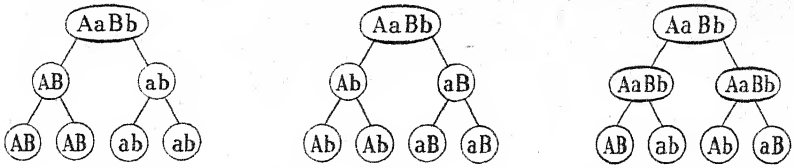
† *Bouloy, Nemours*, 1918.

‡ *Zeit. f. Bot.*, 1915, **7**, 369; 1916, **8**, 353; 1917, **9**, 81; 1919, **11**, 257; *Verhandl. d. Physikal Med. ges. zu Würzburg*, 1920, **46**, 1; *Zeit. f. Induktive Abstammangs und vererbungslehre*, 1922, **31**, 170; *Zeit. f. Pilzkunde*, 1926, **10**, 217.

and that it produced spores which germinated. But the fact that fruit bodies are formed by a monosporous mycelium is no evidence for supposing that the form is homothallic. In a heterothallic species, Kniep considered that the difference between the haploid fruit formed on a monosporous mycelium and a diploid fruit produced on a polysporous mycelium lies in the fact that in each basidium of the haploid fruit body there is only one nucleus when the cell is cut off from the parent subhymenial cell, whereas each basidium of a diploid fruit body has two nuclei. In the haploid basidium the single nucleus divides twice to give four nuclei, while in the diploid basidium there is first fusion of nuclei and this is followed by two nuclear divisions.

The heterothallism of the Hymenomycetes has now been shown to be even more complex than was originally supposed by the investigations of Hanna* and others. Kniep considered that in *Schizophyllum commune* as well as in *Aleurodiscus polygonius*, there are four sexually different kinds of spores, and that in these strains, sex is determined by two allelomorphic pairs of factors which are present in the fusion nucleus of the basidium, and that these segregate on ordinary Mendelian lines. If we represent these factors by the letters Aa and (Bb), then the fusion nucleus of the basidium will have the composition (AaBb). At the second division of the fusion nucleus these factors will segregate as (AB), (ab), (Ab) and (aB) and in this way four different kinds of spores will be formed. Only those spores without a common factor will unite sexually in the mycelial stage. Thus (AB) will unite with (ab), whereas (AB) will not unite with (aB), since they carry the common factor (B). Thus according to Kniep segregation occurs in the first and not the second division of the fusion nucleus. On the other hand, Hanna working with *Coprinus lagopus* found that the spores from any individual fruiting body belonged sexually to four different groups. The basidial analyses, however, showed that though some of the basidia bore spores of two sexes only, a pair of one sex and a pair of another and opposite sex, other basidia bore spores of all four sexes: (AB), (ab), (Ab) and (aB). Since some basidia showed four sexually different kinds of spores on a single basidium, Hanna claimed that segregation occurred in the second and not in the first division of the fusion nucleus. The views of Kniep and Hanna are shown diagrammatically on the next page.

* *Ann. Bot.*, 1925, **39**, 431; 1928, **42**, 379.



(Kniep) (Kniep) (Hanna)

These conflicting views of Kniep and Hanna have been to a certain extent reconciled by Newton,* working with *Coprinus Rostrupianus*. This form, unlike *Coprinus lagopus*, is bisexual, and the basidia bear two kinds of spores only, two of one sex and two of the opposite sex. In this instance, sex is apparently determined by a single pair of Mendelian factors (Aa). In some cases, segregation was found to occur in the second nuclear division as in *C. lagopus*, and in some cases in the first division for both species. In *C. lagopus* it was shown that there are apparently three kinds of basidia, a four-sex type: (AB), (ab), (Ab) and (aB); a two-sex type (AB), (AB), (ab) and (ab); and a further sex type (Ab), (Ab), (aB) and (aB). Of these three types of basidia in the hymenium of any fruit body, 50 per cent are of the first type, and 25 per cent of each of the other two types. Newton has put forward the suggestion, that the results obtained are best explained on the following two grounds: (1) that the two sex factors in the nucleus of each spore are carried upon different chromosomes, one factor on each; and (2) that in some basidia the segregation of the sex factors takes place in the first division of the fusion nucleus of the basidium, and that in the other basidia the segregation of one pair takes place in the first division and of the other pair in the second division.

Heterothallism has now been found in the Ustilaginales (Smut fungi) and in the Uredinales (Rust fungi). In the Uredinales, many of which have complicated life-cycles, it was always considered that in those forms which produced spermatia, these were functionless male organs. It has now been found by Allen† for *Puccinia graminis* and Andrus‡ for *Uromyces appendiculatus* and *Uromyces Vignae* that aecidia do not reach maturity unless spermatia are transferred to the developing aecidia. It is only when the spermatia are transferred in this manner that the aecidia pass into the diploid condition.

* *Ann. Bot.*, 1926, **40**, 105, 891.
† *J. Agric. Res.*, 1930, **40**, 585.
‡ Ibid., 1931, **42**, 559.

Craigie* and later Cummins† have shown that the stimulating action of the spermatia on the developing aecidia is due to living material. Craigie found that when the spermatial exudate was heated to 70° C., its stimulating properties were destroyed. Cummins has criticized this work on the grounds that heating the exudate to this temperature would destroy enzymes, and that it is possible that the enzymes of the exudate might have been responsible for this result. In place of heating, therefore, he filtered the exudate through a small Berkefeld filter. By this means the spermatia were excluded and only the exudate passed through. Pustules of *Puccinia sorghi*, a heterothallic form, treated with filtered exudate failed to form mature aecidia. Thus aecidial formation cannot be explained on the basis of enzyme stimulation, so that Craigie was correct in his ultimate conclusion.

A so-called nutritive theory of heterothallism has been advanced by Gwynne-Vaughan.‡ On this view, it is assumed, for example, that the (+) mycelium may be a saltant, which possesses a gene capable of extracting some essential food substance (A) from the substratum, which is necessary for the formation of ascocarps in such a form as *Humaria granulata*. *H. granulata* is an ascomycete which lacks an antheridium, and in which the ascocarp is developed from an oogonium, and two strains are necessary before the fruit body makes its appearance. It is further supposed on this view that the gene responsible for absorbing (A) does not possess the power of absorbing some equally important and essential food substance (B). On the other hand, if in the (−) strain it be supposed that there is another gene which is able to extract (B) but not (A), then, when the two strains meet, the two essential conditions for ascocarp formation will be present and the fruit bodies are formed. In the Hymenomycetes which have been described as quadrisexual, Gwynne-Vaughan considers that such a conception is difficult to visualize, and that it is equally difficult to imagine a race composed of four different sexes. This of course is perfectly true. On the other hand, it is not difficult to suppose that a race requires four different food substances in preparation for the fruiting period. Thus, if the four characters termed (A), (a), (B) and (b) which these fungi have been shown to inherit on Mendelian lines, each represent the power of rapidly withdrawing from the substrate some

* *Nature*, 1927, **120**, 116, 765. † *Phytopath.*, 1931, **21**, 751.
‡ *Pres. Add. Sect. K. Brit. Assoc.*, 1928, p. 185.

essential food factor, and further, if it be supposed that each spore contains either (A) or (a), and either (B) or (b), the necessary conditions for spore formation will be present when either (AB) and (ab) or (Ab) and (aB) meet.

There may be something in this view, but it is more probable that the presence of four growth-regulators or the formation of four growth-regulators is involved. Recent evidence appears to show that some special factor is formed in each strain, and it is only when either two of these factors meet, or four in the so-called quadrisexual strains, that fruit formation takes place. Thus, it has been shown by Verkaik* for *Mucor Mucedo*, which is a heterothallic form, that zygospore formation can be induced to take place in a culture of a (+) strain alone by introducing a piece of agar taken from a culture of the (−) form, but which is devoid of mycelium. Presumably some substance is formed by the (−) strain in the course of its development which diffuses into the agar, and which is able to induce zygospore formation in the opposite strain. This product, whatever may be its nature, would appear to be a "reproduction hormone," so that a slight modification of Gwynne-Vaughan's views on heterothallism would bring it into line with the newer work on growth hormones, and if Verkaik's work be correct, reproduction hormones.

FRUIT DEVELOPMENT

After fertilization has taken place in the angiosperms, a new phase of development sets in with the formation of the fruit. With regard to the physiology of fertilization itself, little or nothing is known. Whitaker† has observed a rise in the rate of respiration of the eggs of Fucus after fertilization. Whether or no a rise in respiration is a general phenomenon after fertilization has occurred is not known.

In the angiosperms pollination is an essential prelude to fertilization. The pollen grain must first be transferred to the stigmatic surface of the gynoecium. This transference of pollen is brought about either by the agency of insects or of wind. In the gymnosperms the pollen is air borne. In the Bryophyta and Pteridophyta, external fluid water is a necessity for fertilization to take place. Fertilization here is brought about by actively motile spermato-

* *K. Akad. Wetenschappen. Amsterdam, Proc.*, 1930, **33**, 656.
† *J. Gen. Physiol.*, 1931, **15**, 167.

zoids, which need the presence of external fluid water to reach the archegonium. Chemotaxis also plays a part here. It has been found that the archegonia of ferns excrete malic acid which attracts the spermatozoids to them. In the mosses, sucrose appears to play this rôle of attracting the sperms.

The development of the fruit in the angiosperms takes place in a number of different ways. In the simplest cases the fruit is derived from the ovary alone. In a number the style, the floral axis, and even calyx and peduncle take part in fruit formation.

The great morphological changes that take place in fruit formation are accompanied by very definite chemical changes in the composition of the tissues. That chemical changes of considerable complexity occur in such fruits as the apple, pear, strawberry and orange in the course of development is very obvious.

A great number of observations made in recent years have indicated that various edible fruits preserved under storage conditions undergo a number of important changes in composition, and it has been found that the actual conditions of storage are of fundamental importance in the successful handling of the fruit. An extensive examination of the chemical changes that take place in apples from the time of petal fall until the crop has been gathered has been made by Archbold.*

The varieties used were Bramley's Seedling and Worcester Pearmain. The samples were collected at intervals commencing from the time when most of the bloom had fallen and the brown and discoloured stamens were still attached. The various fractions estimated were dry matter, material dried at 50° C. for 44 hours, total nitrogen, sugars (fructose, glucose and sucrose), acid, alcohol-insoluble material and starch.

No starch was found to be present in either variety during the first three weeks of development. Starch was first found in Worcester Pearmain in about 5 or 6 weeks, and in Bramley's Seedling in about 8 or 10 weeks. Following upon the presence of starch, which reached a maximum concentration of 2·01 per cent in Worcester Pearmain and 1·21 per cent in Bramley's Seedling, the concentrations of starch fell. In Worcester Pearmain a little starch was still discovered to be present at the time of the final gathering of the fruit, but in Bramley's Seedling it had entirely disappeared.

* *Ann. Bot.*, 1932, **46**, 407.

The concentration of total solids was found to decrease rapidly during the first three weeks of development, but when the period of starch synthesis commenced the concentration of total solids also increased, while there was but a small increase in the concentration of total solids during the period when the starch was falling in amount.

The total concentration of soluble sugars, glucose, fructose and sucrose was found initially to be approximately 1·0 per cent. In the first phase of development glucose is present in greater concentration than either fructose or sucrose, but later the fructose increases rapidly in concentration and is present in greater amount than glucose. The sucrose also increases in concentration. During the phase of starch increase there is also increase in total sugars at a nearly constant rate, and it is during this period of increase that the large excess of fructose, which is characteristic of the mature fruit, is accumulated. On the other hand, the concentration of glucose remains nearly constant, while the concentration of sucrose does not reach a maximum value in Bramley's Seedling until the time just prior to the final disappearance of all starch, but in Worcester Pearmain the maximum concentration of sucrose was found to obtain while there was still 1·0 per cent of starch present in the fruit. Thereafter the concentration of sucrose remained constant for three weeks before the fruit was collected.

During the last phase of ripening, when starch hydrolysis is taking place, between 80 and 90 per cent of the material entering the fruit is stored as sugar. It is generally considered that the hydrolysis of starch in the fruit of the apple brings about a marked increase in the concentration of sugar, especially in the concentration of sucrose. It was found by Archbold that the amount of sugar produced by hydrolysis of starch is not sufficient to account for the marked fluctuation in the amount of sugar produced, and that the amount of sugar produced from starch is only a small fraction of the total sugar accumulated.

The concentration of alcohol-insoluble material (other than starch) was found to show a continuous fall throughout the developmental period. This rate of fall is rapid at first, but slows up, and during the final phases of maturation of the fruit the change in concentration is very small. The concentration of acid was found to increase during the first three weeks and attained a maximum just after the first appearance of starch. Bramley's

Seedling showed a higher maximum concentration of acid (about 2 per cent) than Worcester Pearmain, in which the maximum concentration value recorded was approximately 1·3 per cent. The acid concentration falls when starch synthesis begins and decreases thereafter throughout the rest of the growing period. The concentration of total nitrogen was found to show a continuous fall.

This investigation shows that in the early stages of the development of the apple fruit, most of the carbon that enters the pulp is stored in an insoluble form. When the fruit sets, 60 to 70 per cent of the total dry material is insoluble in alcohol and only from 6 to 8 per cent is sugar. As development proceeds, however, the amount of carbon stored in the insoluble form decreases, and at maturity when the fruit is ready to be picked, 70 per cent of the dry material of the pulp is now in the form of sugar, and there is only about 14 per cent of insoluble material.

It is equally important to determine whether there are any definite stages in the metabolism of nitrogen in the apple. Archbold was unsuccessful in this direction and was unable to isolate an amount of soluble nitrogen from the apple sufficient to estimate the various nitrogen fractions. She concluded that the nitrogenous substances present in the apple fruit are of protein nature, and that only a small fraction of this protein is water-soluble. Hulme* has been able to overcome the difficulties of extracting soluble nitrogen from the apple and has carried out an investigation into the changes that take place in the various nitrogenous substances of the apple fruit during the period of development from the time of petal-fall to maturity of the fruit.

The variety of apple used was Bramley's Seedling, and the samples of fruit were separated into two fractions, peel and pulp. The nitrogen fractions determined were: Total nitrogen, total soluble nitrogen, amino-acid nitrogen, asparagine nitrogen, free ammonia nitrogen, "rest" nitrogen (i.e. the difference between the total soluble nitrogen—the sum of the amino-acid nitrogen, asparagine nitrogen, and free ammonia nitrogen) and protein nitrogen. This last value was obtained by the difference between total nitrogen and total soluble nitrogen.

As might be expected, the changes in the nitrogen fractions in the peel was found to be small. The results taken as a whole for the pulp showed that there are three phases in the nitrogen

* *Biochem. J.*, 1936, **30**, 258.

cycle. In the early stage there is a rapid increase of soluble nitrogen compounds, especially asparagine. This phase of rapid increase in soluble nitrogen compounds is followed by a period of relative equilibrium between protein and non-protein substances. The last phase is peculiar, for a state of net protein synthesis was found to occur, which is certainly an unexpected state of affairs in a senescent organ.

STERILITY

When a plant fails to produce viable seed it is said to be sterile. The causes of sterility are varied. In the pea, it has been found by Hammarlund* and Håkansson† in Sweden and also by Pellew and Sansome‡ in this country that the factors for violet flowers as opposed to white and for green as opposed to yellow seeds in certain strains of pea are transmitted independently, and presumably must be carried on different chromosomes. Very close linkage of these factors was found in other strains. When the two kinds of strain were crossed, the hybrids were found to be partially sterile, about 50 per cent of the pollen and ovules being ineffective. Cytological investigation showed that the crosses between the two strains have a ring composed of four chromosomes and at meiosis segregation can take place in two ways. If the opposite chromosomes go to the same pole, the gametes will be fertile, whereas if the adjacent chromosomes go to the same pole the gametes will be sterile.

The radish (*Raphanus sativus*) and the cabbage (*Brassica oleracea*) can be crossed when the radish is used as the female parent. The hybrid is intermediate in character between the two parents and almost sterile. A few viable seed, however, are set. Sterility here has also been found to be correlated with chromosomal behaviour. The sterile hybrid has 18 chromosomes, 9 from each parent, and at meiosis the divisions follow a very irregular course. On occasion, however, gametes are formed with 18 chromosomes and union between such gametes gives rise to fertile offspring with 36 chromosomes.

Irregularities in chromosomal behaviour during meiosis will mean that the cells produced by these divisions will have varying and even unusual genetical constitutions. Investigations have shown, for example in wheat hybrids, that in consequence of

* *Hereditas*, 1927, **10**, 303. † Ibid, 1931, **15**, 17. ‡ *J. Gen.*, 1931, **25**, 25.

this fact some of the pollen and ovules do not develop normally. Similar results have been obtained for the Jimson weed or thorn apple, *Datura stramonium*. The question of pollen-tube growth in this genus has been especially carefully investigated, and it can be stated as a general rule that irregularity in chromosomal behaviour will lead to some degree of sterility.

There is a second phenomenon of sterility known as incompatibility, the failure of crossing to take place, that must be considered here. Some plants are self-sterile. This is the best method of preventing autogamy in a hermaphrodite flower. Incompatibility, however, is a complex phenomenon.

Fertilization in the flowering plants is brought about by the pollen containing the male gametes with the haploid number of chromosomes (n) being placed on the stigma of another plant. On the stigma the pollen sends out a pollen-tube which grows through the somatic tissues of the style, which contains the diploid number ($2n$) of chromosomes, to the ovary. Here the two male gametes are discharged by the bursting of the end of the pollen tube, and one of the gametes fuses with an egg-cell with n chromosomes to give an embryo with $2n$ chromosomes, while the other fuses with two other female nuclei to give the endosperm which has a chromosome complement of $3n$.

In certain instances it has been found that the success of these processes is influenced by Mendelian factors and by the chromosome number of the tissues involved. Thus pollen-tube development may be inhibited and fertilization does not take place. It has been shown by East and Mangelsdorf* for Nicotiana, that there are a series of multiple allelomorphs involved, S_1, S_2 and S_3, which determine whether the pollen-tubes will grow fast enough in the style to effect fertilization. Thus a pollen tube having the factor S_1 will not grow normally in the style of a plant also with S_1.

In *Cytisus Laburnum* it has been found by Jost that the pollen will not germinate unless the stigma has first been wounded. This plant is pollinated by the bee, and in its visit to a flower it inflicts slight wounds on the stigma which enable the pollen to germinate. Charles Darwin recorded a number of cases of self-sterility among orchids. If the pollen of *Oncidium flexuosum*, for example, be placed upon the stigma of the same flower, or even on that of another flower of the same plant, it turns brown and dies within five

* *Genetics*, 1926, **11**, 466.

days, whereas pollen from a different plant on the same stigma is perfectly fresh.

Why the pollen should not develop or the tubes fail to grow is not known. It has been suggested by Correns that it may be due to the presence of inhibiting agents. Jost, however, is not in agreement with this view, and considers that some essential growth factor must be absent.

INBREEDING AND HYBRID VIGOUR

Among so-called "practical" breeders, inbreeding has always been looked upon with suspicion. In general, close inbreeding for successive generations, e.g. brother to sister matings, leads to degeneration of the stock. In plants, as well as animals, close inbreeding for several generations frequently brings in its train lack of fertility and vigour in the offspring.

Inbreeding, however, does not always lead to degeneration in this way, so the rule that inbreeding leads to degeneration is not a universal one. A number of domestic breeds of animals and plants have been closely inbred for years without degeneration setting in. Jersey cattle and the Saint Bernard dog are examples in which inbreeding apparently does not produce harmful results. Pigs, on the other hand, quickly degenerate when inbred. There is one example where close inbreeding has resulted in an actual improvement in the stock. In a certain race of albino rats which were inbred for more than twenty generations by brother to sister matings, the average weight, fertility (number per litter) and the life span of the individual had increased.

In the great majority of cases, however, it must be admitted that inbreeding is definitely harmful and leads to degeneration of stock. It is equally true that a cross between two separate strains usually produces strong and vigorous offspring, or as the "practical" breeder would say "fresh blood" has stimulated the race.

It is clear that inbreeding *per se* is not harmful, because a number of varieties of plants and animals can be inbred without harmful results, and the experiment described above with albino rats actually led to an improvement in the race. The question arises as to the explanation of these apparently contradictory facts. In the first place inbreeding reduces the number of ancestors con-

tributing to a race. In the second place it is well known that a strain which has been inbred for a long time is more homogeneous and less variable than one which is cross-bred and mixed. In other words, an inbred race tends to sort itself out into a number of races, each pure-breeding and homogeneous. Since the hereditary mechanism is composed of particulate factors or genes, which may be either dominant or recessive, continuous inbreeding will tend to bring the recessive genes to the surface. They will be brought together in pairs, freed of their dominant alternatives and thus show themselves. On the other hand, cross-breeding will tend to conceal the recessive because the chance of their being submerged by dominants will be increased. Evidently the relative merits of inbreeding and out-breeding will depend on the nature of the recessives carried by a particular strain. If the recessives are harmful, inbreeding will obviously be bad for the strain, whereas if the recessives are good, inbreeding will produce desirable results.

East and E. M. Jones have deliberately employed inbreeding to improve maize stock. The plants were intensively inbred for twelve or more generations. As a result, the recessives revealed themselves and the plants separated into a number of true-breeding lines. Some remarkable results were obtained, some of the strains were stunted, some were infertile, some susceptible to fungal attack and so forth. The most desirable strains were picked out and recrossed with one another and a new breed obtained with the desirable characters of the original strain, but with the hidden, undesirable recessives removed.

It has already been mentioned that crosses between unrelated strains frequently lead to a more vigorous offspring. As long ago as 1760 Kölreuter, who made a number of crosses between different species and varieties of plants, often found that the hybrid grew more vigorously than either parent. Mendel in his work with peas, found that the hybrids from the cross between a tall and dwarf variety were frequently taller and showed greater luxuriance in growth than the tall parents.

This phenomenon of greater vigour in the offspring than the parents is known as *hybrid vigour* or *heterosis*. Ashby* has attempted a physiological analysis of maize in order to determine the underlying physiological causes of heterosis. He has suggested that hybrid vigour may be due to one of the four following reasons

* *Ann. Bot.*, 1930, **44**, 457; 1932, **46**, 1007.

or to a combination of these circumstances: (1) the hybrid possesses more meristematic centres so that it is able to develop more tillers and leaves; (2) the photosynthetic mechanism of the leaves of the hybrid is more efficient than that of the parents; (3) the hybrid has a larger embryo than either of the parents, and on this account has a higher initial capital at the time of germination; (4) the falling off in the S-shaped curve for growth may occur at a later period in the hybrid than in either of the parents.

In the first experiments two inbred strains of maize were used, one of which possessed a light blue grain and the other a white starchy grain. The hybrids obtained by crossing these two strains were taller and possessed larger leaves than either parent. Such factors, as mean dry-weight, respiration rate and assimilation rate of hybrids and parents were determined.

The first interesting point that emerged was that the relative growth rate of the hybrids was the same as that of one of the parents. It is therefore evident that hybrid vigour in this instance cannot be due to an increase in relative growth rate of the hybrid over the parents. It was found that the hybrid embryo was larger and heavier than either parent. At germination, then, the hybrid will commence with the initial advantage of having a larger capital than its parents, and this advantage is retained throughout the life-cycle.

After germination the hybrid was no more vigorous than its parents as measured by such physiological processes as respiration and photosynthesis. It would appear from these results that relative growth rate is inherited from one parent as a dominant Mendelian character. Thus the efficiency index, r, in the equation given for compound interest by V. H. Blackman for plant growth, $W_1 = W_0 e^{rt}$ (see Chapter XV), is the same as in one of the parents but not of the other.

Significant differences were found between the embryo weights of reciprocal crosses, although these reciprocal crosses had the same genetical constitution. This difference is presumably due to maternal influences during the development of the embryo and before the seed enters upon the resting period.

Theories regarding hybrid vigour have held that it is due to some kind of stimulus at the time of fertilization. This view, however, need not be considered any further, for it is entirely wrong. It was shown by Keeble and Pellew,* that when two inbred

* *J. Gen.*, 1910, **1**, 47.

strains of peas were crossed, the hybrids were more vigorous than the parent strains, and they put forward the hypothesis that heterosis is the result of the meeting of complementary dominant factors. On this suggestion the hybrid vigour from reciprocal crosses of maize should have been the same, but according to Ashby's results the embryo weights of reciprocal crosses differed significantly, and he has suggested that maternal influences must play a part in the matter.

On *a priori* grounds it would be expected that a hybrid showing heterosis would have a greater efficiency index than the parents. This, however, in maize, is inherited like a single Mendelian dominant character, and hybrid vigour is apparently nothing more than keeping up the initial advantage obtained by the possession of a larger embryo through the grand period of growth, but although the capital is larger in the hybrid, the rate of interest is the same as in the parents.

These results have been obtained for maize. Whether they are of general application still remains to be determined. Ashby* has investigated the problem of hybrid vigour in the tomato and has put forward the same suggestion as for maize, namely, that the hybrid has a heavier embryo. This work, however, is still in the preliminary stage and it will be of interest to see whether the final results bear out the results found for maize.

* *Ann. Bot.* (New Ser.), 1937, **1**, 11.

CHAPTER XVII

IRRITABILITY AND PLANT MOVEMENTS

It is a well-known fact that all plants are able to show some power of movement. If, for example, one of the higher green plants be laid in a horizontal position, it will be found that after the lapse of a certain time, the shoot will describe an upward movement, while the root will direct itself downwards. The plant has reacted to the stimulus of gravity, and the shoot is said to have performed a negative geotropic curvature, whereas the root has performed a positive geotropic curvature. Actually, the upward curvature of the shoot does not take place at the apex but some distance from it, and in executing this movement the plant has done work. The work, however, that has been carried out by the plant has only been performed at a certain particular part, the point of curvature, for the distal end of the shoot is elevated into the air in a purely passive manner.

The active movements that are exhibited by plants are set in motion by a number of different stimuli, and these stimuli may be either external or internal in nature. In the case of plants which grow in fixed positions we have only movements of definite organs to consider. On the other hand, with unicellular organisms, such as Chlamydomonas, various zoospores and spermatozoids, we have to consider the active locomotory movements of the entire organism.

The various movements described by plants are due to the fact that the protoplasm is sensitive to stimuli and reacts accordingly. The apex of a higher green plant in a state of active growth does not grow upwards in a straight line. As the growing shoot elongates a complicated spiral is performed in space, a phenomenon termed *circumnutation*. This is one kind of movement shown by plants. In other cases differential growth takes place on opposite sides of a plant member, which may result in a definite curvature. An example of this type of curvature has already been described above for the response of a shoot and a root under the stimulus of gravity. When a plant organ ceases from active growth, nutation and growth movements also come to an end. At the same time it must be borne in mind that even when active growth has been completed, the power of movement is still retained in

certain cases of particular organs. In many of the Leguminosae, special motor-organs are present, known as pulvini. These are found as swellings at the base of petioles and leaves, and are specially adapted for executing various movements although the growth of the petiole has ceased. Furthermore, in certain instances growth may be restarted under the influence of a stimulus. An example of this is to be found in the nodes of grasses which are stimulated into fresh growth under the stimulus of gravity.

It is obvious that a plant organ which reacts to a stimulus must be able to perceive that stimulus. In this connection it is necessary to say a word with regard to what is known as *tone* or *tonus* of the reacting protoplasts. The response shown by an organ depends upon the condition of the protoplasts of the cell. It sometimes happens that when a plant is submitted to a particular stimulus no response is shown. The response of an organ is said to be dependent upon its condition of tone, and this in turn is dependent upon the previous conditions to which the organ has been submitted and also upon its stage of development. The leaves of *Mimosa pudica*, the so-called "sensitive plant," react in a very characteristic manner to touch or wounding (see under the section, Seismonic Irritability). It has been found that after a leaf has been stimulated a number of times, no further response is shown. The plant is said to have passed into a state of *rigor*. The cells of the leaf have lost tone and are no longer in a condition to respond to the stimulus of wounding, and it is only after an interval of time that tone is again recovered and a reaction shown.

Changes in tone may be brought about by the action of anaesthetics, such as chloroform and ether. In low temperatures (0–2° C.) Czapek found that the roots of Lupinus seedlings laid horizontally did not show geotropic curvature even after 18 hours. When, however, the roots were removed to a more favourable temperature a positive geotropic curvature was described. Lack of oxygen is said to suppress response to stimuli. In each of these cases the existing tone of the cells has been affected and no response is given. The stage of development may also influence the response. Stems and leaves when in the bud stage are said to be insensitive to phototropic and geotropic stimuli.

It is a difficult matter to define satisfactorily what is meant by the term stimulus and it is possible to classify plant responses to various stimuli in a number of ways. Thus plants respond to such

stimuli as heat, light, gravity, etc., and it would be possible to classify the movements exhibited according to the nature of the stimulus. It is, however, better to employ the nature of the reaction as a basis of classification of plant movement. In any case, the fact that a plant or plant organ shows a response to a particular stimulus means that the protoplasts possess specific irritability, although the response shown may and does vary with different plants. Movements which result from the effect of some external stimulus, such as light or gravity, are termed *induced*, *aitiogenic* or *paratonic* movements, whereas movements which result from internal stimulation are called *autonomic*, *autogenic* or *spontaneous* movements.

The reactions shown by plants in response to stimuli may result in alterations in length, or in bendings, twinings or twistings of the organs. The alterations in form induced by a stimulus result in a part at least of the organ taking up a new position, or occupying a new relationship to the remaining parts of the plant. When a fresh position shows a relationship to the direction of the applied stimulus, the movement is termed a *tropism*. On the other hand, when the stimulus is diffuse and has not been applied in any one particular direction, or the response induced in the plant organ bears no relation to the direction of application of the stimulus, but is determined by the activity of the plant itself, the movement is termed a *nastic* movement. The term *taxis* is employed to indicate locomotory directive movements of whole organisms. Thus, just as we speak of the response of a plant organ to unidirectional light as phototropism, so we can speak of the directional locomotory activity of a unicellular form to unidirectional light as phototaxis.

It was at one time considered that there must be a certain minimum "threshold" difference which must be passed before a plant organ in a sensitive condition could distinguish between differences in intensity of some stimulus applied from opposite directions. It was supposed that a difference in intensity of the stimulus over and above this threshold value led to a greater physiological disturbance of the irritable member and that the after effects were also greater. Thus a large number of investigations were made to show for plants that the response is proportional to the logarithm of the inducing stimulus, a result known as Weber's law. Recent investigations, however, have shown that Pfeffer's original conception of a stimulus as a releasing mechanism

is the more productive line of attack. On Pfeffer's view there is no relationship between the applied stimulus and the response made by the stimulated plant. The stimulus appears merely to release a certain chain of reactions. Pfeffer's idea can be likened to the action of a trigger of a gun. If the trigger of an unloaded gun is pulled there is naturally no response, but when the trigger is pulled of a loaded one, there is an explosion, and the magnitude of the explosion does not depend upon the amount of pressure that is placed upon the trigger, but upon the amount of powder in the cartridge.

There are certain terms employed in the study of the irritability phenomena exhibited by plants which must be defined. For example, organs which in their equilibrium position lie in the line of an applied stimulus are said to be *orthotropic*, when the organs place themselves at an angle to the line of direction of a stimulus they are said to be *plagiotropic*. In many cases the response of an organ does not take place until some time after the stimulus has been removed. Thus, although perception of the stimulus and the resulting response are linked reactions, they are separated by a time interval. Such terms as *reaction-time*, *presentation-time* and *relaxation-time* have been used to express the time-relations of plant movements to stimuli. Reaction-time is defined as the time taken for a visible response to be given by a plant organ that has been under constant stimulation. The time during which a plant organ must be submitted continuously to a particular intensity of stimulus for a visible response to be made is termed the presentation-time. The time taken for a stimulation to subside is called the relaxation-time.

GEOTROPISM

The phenomenon of movement due to the action of gravity is termed *geotropism*. Under the force of this stimulus primary roots tend to grow downwards in the direction of the force, they are said to be positively geotropic, whereas primary stems tend to grow in the opposite direction, i.e. they are negatively geotropic. If a growing plant be changed from the vertical to the horizontal position, the root-tip soon begins to bend downwards and the tip of the stem grows in the upward direction.

The geotropic response of lateral shoots and lateral roots is less strong than the primary organs, and these organs do not assume a vertical position but extend at a definite angle from

the primary root or shoot. They are *plagiogeotropic*. Laterals of a higher order are practically or entirely insensitive to the stimulus of gravity (*apogeotropic*). This decreasing sensitivity to gravity of the lateral root system is of definite biological advantage to the plant. The spread of the root system through the soil is facilitated by the diminished geotropic irritability of the lateral roots of the second and third order. In the same way, the decreasing geotropic irritability of the aerial shoots of secondary and higher orders allows of the leaves, for example, to become better placed for receiving light for assimilation, than would be the case if all the members reacted similarly to gravitational stimulus.

The aerial roots of such plants as the Ivy, Orchids and Aroids are insensitive to gravity, but respond to phototropic stimulus. Horizontally growing rhizomes are diageotropic, and so are certain root-stocks, such as *Sparganium ramosum*. On the other hand, the rhizomes of Yucca which grow downwards are positively geotropic.

The sporangiophores of *Phycomyces nitens* and *Mucor Mucedo* show negative geotropism. The stipe of some of the Hymenomycetes has also been shown to give a negative geotropic curvature. It has been found by Buller that in the case of the Hymenomycetes the geotropic response that is exhibited is in the nature of a gradual adjustment. The growing stalk swings across beyond the vertical line, then direction is changed, and the stalk swings back and passes the vertical two or three times before it comes to rest.

In certain cases it has been found that the geotropic response of a given plant organ may change in the course of development. The peduncle of the poppy before the flower bud has opened is positively geotropic, but gradually becomes negatively geotropic and straightens as the flower expands. The behaviour of the young stems of twiners to geotropic stimulus is peculiar, and is considered in a separate section (see below).

It has already been mentioned that lateral shoots and roots are plagiotropic. It was shown by Bruck* that when the terminal 2 mm. of the primary root was cut off, so that elongation of this organ was prevented, the lateral roots just above the wound no longer showed plagiotropism but became positively geotropic. He also showed that when the tip of the conifer *Abies pectinata* was broken off, one of the lateral branches became negatively geotropic.

* *Zeit. Physiol.*, 1904, **3**, 486.

It was first shown by Sachs in 1873 and later by Baranetzsky that when a radial shoot is laid in a horizontal position (Fig. 40) the first sign of negative geotropic curvature commences in the more actively growing apex of the shoot. The upward curvature of the shoot is continued and passes beyond the vertical, although it is more and more withdrawn from the influence of gravity. The fact that over-curvature is shown before the vertical position is reached, is partly due to the persistence of the geotropic induction, and partly owing to the lower regions being inclined to the vertical, and therefore continuing to curve. The apex of

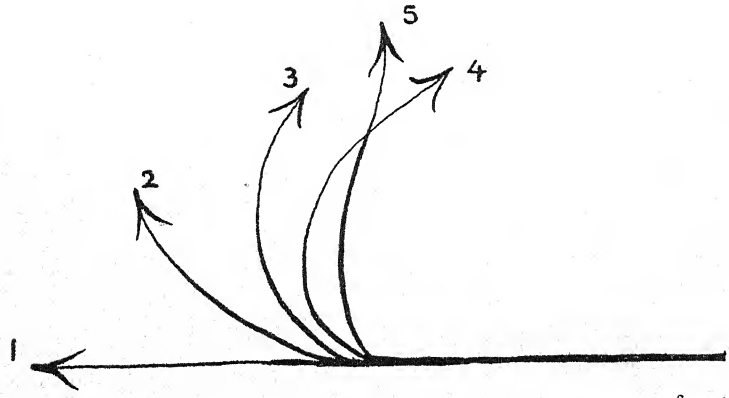


FIG. 40.—Diagrammatic representation of negative geotropic curvature of a stem. Note that over curvature is shown before the vertical position is assumed.

the stem finally attains the vertical position and only the basal portion remains curved.

Tropic curvatures which are produced by growth cease when growth has come to a standstill. Nevertheless, various mature organs are able to give tropic responses. This is especially noticeable in leaves which possess pulvini. Here, after the leaf is apparently fully grown, geotropic and phototropic responses are still shown for many weeks and even in some cases for months. Eventually, however, this power of reaction is lost in every case.

In grasses, when the stem is laid in the horizontal position, negative geotropic curvature takes place through the activity of the nodes, for the internodes are insensitive to gravitational stimulus. Under the stimulus of gravity growth is recommenced on the lower sides of the nodes and upward curvature takes place. But even in grasses the nodes are unable to retain this

power of fresh growth indefinitely, and a grass-node is only able to perform one or two geotropic curvatures. The nodes of certain other plants, such as *Dianthus bannaticus* and *Tradescantia fluminensis*, also show this power of renewed growth under the stimulus of gravity. In general, however, experiments have shown that it

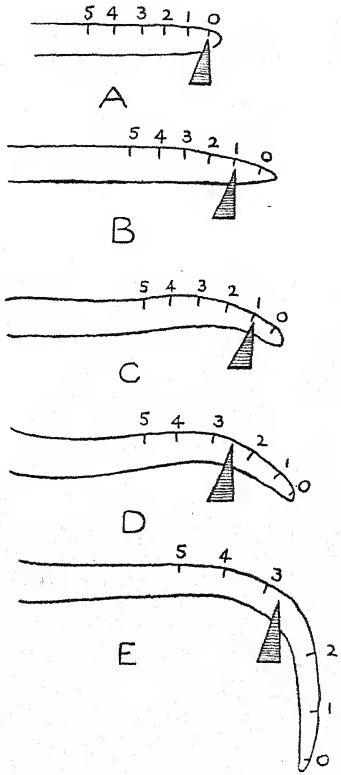


FIG. 41.—Positive geotropic curvature in a root. For description see text.

is only those regions capable of growth that perform curvatures. For this purpose roots or shoots marked with indian ink at equal distances are much used, and it can be shown that geotropic curvature takes place by the greater growth of the lower side under the gravitational stimulus (Fig. 41).

THE MECHANISM OF GEOTROPIC CURVATURE

In 1806 T. A. Knight carried out his well-known experiment of attaching young seedlings to a wheel, which was then rapidly

rotated in a vertical plane. As a result, he found that the roots of the seedlings grew out towards the periphery of the wheel, whereas the shoots grew towards the centre of the wheel. By this means Knight equalized the one-sided action of gravity upon the plants and at the same time submitted them to the action of centrifugal force. Stems and roots in this case behaved in the same way towards centrifugal force as they do towards gravity. In a second experiment Knight allowed the wheel to revolve in a horizontal plane so that gravity and centrifugal force acted upon the material simultaneously, but in different directions. Thus a composite effect was produced and the roots grew outwards and obliquely downwards, whereas the stems grew inwards and obliquely upwards. It is evident that the vertical orientation in space of shoots and roots is primarily determined by gravitational force.

It was at first thought that the downward curvatures shown by roots when they were placed in the horizontal position was solely due to their own weight. Later it was shown that geotropic curvature can only come about through growth. In any case, such a purely mechanical interpretation will not account for the negative curvature of shoots and plagio and diageotropic reactions of plant organs. It was suggested by Knight that geotropic curvatures were caused by the redistribution of different substances of varying specific gravity in the plant, but as far as the geotropic behaviour of the root was concerned, he appears to have considered that mass-attraction by gravity was sufficient to bring about curvature. The negative geotropic response of shoots was explained by Knight as being due to denser nutrient sap collecting on the under surface of the horizontally placed shoot, and as a result the lower side of the shoot grew more rapidly than the upper and the shoot curved upwards.

The theory, independently put forward by Němec and Haberlandt, that the geotropic excitation in plant cells is due to falling starch-grains, or possibly, in certain cases, to other solid bodies, such as calcium oxalate crystals, must now be considered. On this theory, the perception of gravitational stimulus by plants is the same as that found in the balancing organs of certain animals. These balancing organs are known as *otocysts* or *statocysts*, and the solid bodies in the otocysts are termed *otoliths* or *statoliths*. The starch-grains present in plant cells are considered to serve the same function in plants for the perception of gravitational

stimulus. In the higher plants, Haberlandt considers that special gravitational sense-organs are present, which are made up of a number of sensory cells or statocysts. Each individual statocyst is considered to be made up of a varying number of easily movable starch-grains, and an "ectoplast" which is sensitive to the pressure of these statoliths. If an organ in geotropic equilibrium be considered, the starch-grains will be on the physically lower portion of the ectoplast and as a result there will be no geotropic perception by the ectoplast leading to a responsive movement. If now the geotropic equilibrium be upset, for example, by laying a root horizontally, the starch-grains will fall against that part of the ectoplast which is the new physically lower side, and produce a new stimulation and a geotropic curvature, with the result that the organ is brought back to its former condition of equilibrium.

In the root, the perceptive and responsive regions to gravitational stimulus are distinct. It was shown by Charles Darwin that no geotropic curvature takes place in roots which have been decapitated and then placed horizontally. On the other hand, if the roots be first of all laid horizontally for longer than the presentation-time and are then decapitated before any visible curvature has occurred, a geotropic response is shown. It is therefore evident that the tip is necessary for the perception of the stimulus, but not for the response. It should be mentioned that this experiment to demonstrate that perceptive and responsive region to gravity is distinct is not altogether convincing. Local injury will cause a tropic response in the growing-points of roots and a curvature takes place away from the injured side. This is known as a *traumatropic* curvature. To overcome this objection, Czapek grew seedlings with the tips of the roots enclosed in small glass slippers, which were placed either vertically or at various angles to the vertical. He established the fact that whatever angle the remainder of the root formed with the vertical, geotropic curvature did not take place as long as the tip of the root was pointing vertically downwards. If the tips were at an angle with the vertical, then geotropic curvatures were brought about, even if the remainder of the growing region of the root were vertical. In this connection of the separation of the geotropically perceptive region and the region of curvature, it was established by Francis Darwin that in seedling grasses it is the apex of the shoot which is the perceptive region and it is the

position of the shoot-apex which determines whether geotropic curvatures will occur.

A formidable amount of experimental evidence was put forward by Haberlandt and Němec in support of the statolith theory of geotropic response. It was found by Francis Darwin that there is a diminution of geotropic irritability in plants which have been kept at high temperatures as the starch disappears, while Němec discovered that if the starch-bearing columella of Lupinus roots were removed, geotropic curvature only resulted after 20 hours, and that by this time the starch grains had reappeared. It was also found by Němec that a root does not show geotropic curvature for a long period if the root-cap has been removed, and geotropic curvature only occurs when falling starch-grains reappear in the wound callus. Experiments in which tissues have been wounded are not very convincing, and Němec's work with uninjured roots of *Vicia Faba* is to be regarded with less suspicion. The roots of *V. Faba* were embedded in plaster of Paris and as a result growth was completely inhibited and the starch-grains in the root-cap were entirely hydrolysed and disappeared. This condition of the starch in the root-cap occurred in a week. When the roots were removed from the plaster of Paris they recommenced growth, but failed to show geotropic response. It was not until starch-grains reappeared in the root-cap that the roots responded to gravitational stimulus. Even this experiment is open to the criticism that the roots were embedded for so long in plaster of Paris that temporary injury may have been caused to the protoplasts of the sensory cells. This objection, however, does not apply to some experiments made with the onion. Onion bulbs that had been stored for several years were allowed to germinate. The roots that were first formed showed a strong hydrotropic response, but were insensitive to gravity. Examination of the root-cap showed that there were no starch-grains present. After several days' growth, the roots responded to gravitational stimulus and it was now ascertained that starch-grains were present in the root-caps.

Haberlandt has examined such plants as *Linum perenne*, *Capsella Bursa pastoris* and others in this connection. By prolonged exposure to cold, the starch in the starch-sheath of the shoots disappeared. Stems which had been completely deprived of starch were then laid horizontally for 2 to 2½ hours at a temperature of 17 to 20° C. and subsequently rotated on a klinostat. No geotropic curvature

was shown. Under laboratory conditions starch reappeared in 24 hours and the plants responded to gravity when laid horizontally for 2 to 2½ hours. Still further evidence for the statolith theory was obtained by Buder using the roots of *Lepidium sativum*. The roots were first of all laid horizontally for 12 to 15 minutes. At the end of this time all the starch-grains had fallen to the lower side of the horizontally laid roots. The roots were next turned through an angle of 180° and left in the new position for exactly the same time as in the first position. No geotropic response was shown, although the statoliths were present on one side of the roots, for the stimuli acting in two opposite directions had neutralized one another.

Haberlandt has distinguished a number of different kinds of statolith-apparatus in roots. In ordinary roots the region of perception is present in the tip and the response is shown by the sub-apical growing zone. The central portion of the root-cap is considered to be the actual zone of geotropic perception and the cells of this particular zone contain starch-grains. In *Selaginella Martensii* starch-grains are absent from the root-cap, but are present in the inner layers of the periblem, and this is considered to be the perceptive region for this plant. *Trianea bogotensis* is very similar to *Selaginella Martensii*. If the tips of the roots of *Trianea bogotensis* be removed geotropic reaction is still shown.

In secondary and higher orders of lateral roots the statolith apparatus is said to be very much reduced. The aerial roots of such plants as *Arum maculatum*, which show no geotropic response, contain no starch-grains in their root-caps, or if starch-grains be present they are few in number and very irregularly distributed.

In stems and leaves Haberlandt has also distinguished a number of different types of statolith-apparatus. Normally the statolith-apparatus is located in the starch-sheath of stems, as well as inflorescence axes, peduncles and pulvini. In the next type, odd cells of the starch-sheath contain no starch, and on occasion a whole series of these cells devoid of starch may be present. Other and more complex types are the statocysts of *Urtica dioica* in which the starch-sheath is still further disrupted and the special "sickle-shaped" groups of cells containing starch-grains which are to be found in the nodes of grasses. In *Thalictrum flavum* the statolith-apparatus is located in the primary medullary rays.

An elaborate examination of the statolith-apparatus in dicoty-

ledons, monocotyledons and conifers has been made by Hawker.* In *Lathyrus odoratus* in which the cotyledons are hypogeal it was found that in seeds which had been soaked for 24 hours the cotyledons were packed with starch-grains of large size (25 μ), while in the remainder of the embryo the grains were small (2 to 3 μ). This non-movable starch present in these structures was termed by Hawker "embedded starch" to distinguish it from statolith starch. When the plumule of the young seedling is 2 mm. in length, the starch-sheath is packed with embedded starch, and it is also present in the single layer of cells surrounding the leaf traces. When the young shoot has increased in length, the grains are still small, but are now free to fall, and the amount of embedded starch decreases with the growth of the epicotyl. When the shoot is 6 cm. long, embedded starch is confined to the apical bud and point of the epicotyl. At this stage, although the starch-sheath is well developed, the statocysts (cells containing statoliths) are not completely differentiated. Some of the starch-grains are free to fall, while others remain attached to the upper and lateral walls. This stage is termed the *zone of development.* Below this zone there is another region corresponding to the region of geotropic curvature. Here the starch-grains are well developed and this is called the *zone of efficiency*, and it is here that the geotropic stimulus is perceived. The statocysts form a single layer of cells surrounding the vascular cylinder. Beneath the zone of efficiency, yet another region is distinguished, the *zone of disintegration.* In this zone the starch-grains have lost their power of development and have become embedded in the lining of cytoplasm of the cells. The grains are split up into smaller ones and may possibly serve a nutritive function.

A number of hypogeal seedlings were also examined, such as *Vicia Faba*, *Aesculus Hippocastanum*, Quercus sp. *Citrus paradisi*, etc., and in all essentially the same arrangement was found to obtain.

In the root, statolith starch was found to first make its appearance in the root-cap.

In epigeal seedlings, such as *Ricinus communis*, a considerable amount of embedded starch was found in the cotyledons and hypocotyl. As the growth of the seedlings proceeds, this embedded starch decreases in amount very rapidly. It disappears first of all from the base of the hypocotyl, and lingers for some time at the apex. As in *Lathyrus odoratus*, Hawker was able to distinguish

* *Ann. Bot.*, 1932, **46**, 121.

the zone of development, of efficiency and of disintegration. The statocysts were located in the starch-sheath of the hypocotyl, and with growth in length of this organ an increasing region at the base was found to become insensitive to gravitational stimulus, and examination showed that no statoliths were present. In *Beta vulgaris*, which does not normally form starch in photosynthesis, statolith starch was found to be present in the hypocotyl.

In all, epigeal seedlings from 42 species from different families were studied and the morphology of the statolith-apparatus was found to be essentially uniform in the hypocotyl of dicotyledonous seedlings, namely, a single layer of cells, the starch-sheath. As is to be expected, individual variations were found.

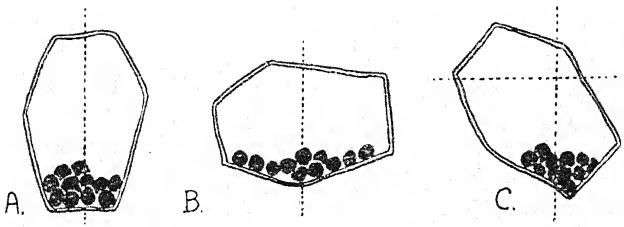


FIG. 42.—Statocysts and statolith starch. (After Hawker.)

For example, in *Euphorbia Peplus* the ring of statocysts is not complete.

A study of the statocysts was also made in this investigation. In transverse sections these cells are not isodiametric. A statocyst in position A (Fig. 42) when stimulated will have less cell wall and less sensitive layer of protoplasm and therefore will be less stimulated than in position B. Furthermore, the starch-grains will have a greater distance to fall in A than in B. Hence the statocyst in position B will be more efficient than in position A and a shorter period of stimulation will be sufficient to bring about response in that plane than in any other. Again, if the long diameter of the statocyst is at an angle of 30° to 60° to the cotyledonary plane (C), it will still be equally as efficient in either the cotyledonary or intercotyledonary plane.

From the evidence available it is probable that movable starch-grains play an essential part in the early events that lead up to geotropic curvature. They probably function in some way as a releasing mechanism. The question arises here, How is actual curvature brought about?

It was found by Snow* that when he decapitated the roots of *Vicia Faba* at a distance of 2 mm. from the vegetative point and then replaced the tips with gelatin, geotropic curvature occurred, whereas the decapitated stumps when gravitationally stimulated gave scarcely any response. It was further discovered by Snow that when the root-tips were killed by immersion in boiling water and then replaced on the stumps with gelatin, no geotropic curvature was exhibited after stimulation. It is thus evident that in *V. Faba* the perceptive zone of geotropic stimulus is located in the root-tip and that if the root-tips are killed they lose their perceptive power, and further, that the stimulus can pass or diffuse through a layer of gelatin.

Cholodny† in a series of investigations on *Zea Mays* also showed that the geotropic response of the decapitated roots of this plant could in large measure be restored by replacing the tips upon the stumps. He next decapitated roots and instead of replacing the tips he tried the effect of attaching the tips of the coleoptiles. The surprising result was obtained that the roots now responded more strongly to gravitational stimulus than if their own tips had been replaced. It will be recalled that the coleoptiles of grasses are negatively geotropic, yet when the tips are placed on the stumps of decapitated roots a strong positive reaction is shown. This effect was explained by Cholodny as being due to the different physiological nature of the two stumps and he concluded that their growth rates were oppositely influenced by the same substance (auxin) which diffused into them from the coleoptile tip. Thus the influence of auxin on the coleoptile was to cause an acceleration of growth, whereas in the root it brought about retardation of growth. Cholodny was able to support this contention by showing that the growth rates of decapitated roots and coleoptiles were influenced in opposite directions, whether root-tips or coleoptile tips were placed upon the stumps. Thus, if he placed root-tips back upon root stumps or coleoptile tips on root stumps there was always a retardation of growth. On the other hand, if he replaced coleoptile tips or root-tips on coleoptile stumps there was an acceleration of growth. This investigation fully confirms the earlier work of Snow on *Vicia Faba* and shows that in the root-tip, as in the case of the apex of the coleoptile,

* *Ann. Bot.*, 1923, **37**, 43.

† *Ber. deut. bot. Ges.*, 1924, **42**, 356; *Jahrb. f. wiss. Bot.*, 1926, **65**, 447; *Planta*, 1928, **6**, 118; 1929, **7**, 461.

there is some substance which markedly affects the growth of this organ.

Cholodny's theory has been accepted by Keeble, Nelson and Snow,* who have further investigated the matter. They first of all showed that if a root were geotropically stimulated, and then decapitated, and the tip placed upon the stump of an unstimulated root, a reaction took place. In the second place, if a root were first of all decapitated, which normally would show no response, and then geotropically stimulated, was then headed with the tip of an unstimulated root, curvature took place.

The suggestion has been made by Keeble, Nelson and Snow that the root-tip is able to bring about a geotropic reaction in two ways. Firstly, the stimulus can be transmitted from the tip to an unstimulated stump, and secondly, some stimulus can pass to the stump from an unstimulated tip which also makes it sensitive to gravity.

According to Went,† the geotropic curvature of the coleoptile of the oat is due to a redistribution of auxin. When this organ is placed in a horizontal position the redistribution of auxin takes place in such a way that its concentration upon the lower side of the elongating region of the coleoptile is greater than on the upper side. As a result of this higher concentration of auxin on the lower side, the cells of this region are stimulated into greater growth and upward curvature follows.

On the supposition that there is a redistribution of auxin, not only in the tip but also in the elongating region, the two different ways in which tips reheaded on to stumps cause these structures to react can be understood. Keeble, Nelson and Snow suggest that, in the first place, auxin diffuses out in unequal concentration on the two sides from a stimulated tip, and in this way brings about curvature in an unstimulated stump. In the second place, they consider that auxin diffuses out equally on all sides from an unstimulated tip, but when the auxin passes into a stump which has been submitted to gravitational stimulus, it is redistributed in some way which is not understood, but which results in the auxin reaching the upper and lower sides in different concentrations. They agree with Cholodny that the root stump reheaded with a coleoptile tip which shows positive geotropic curvature and grows downwards and not upwards, should be

* *Proc. Roy. Soc. (Lond.)*, 1929, **105B**, 493; 1931, **108B**, 537.
† *K. Akad. van Wetenschappen Amsterdam Proc. Sect. Sci.*, 1926, **30**, 10.

interpreted as being due to a retardation of growth brought about under the influence of the coleoptile stump.

Hawker* has been able to show that there is a redistribution of auxin in the roots of *V. Faba* under geotropic stimulus. The seeds were germinated, and when the radicles were from 6 to 12 cm. in length, two-thirds of the total number of roots were placed in a horizontal position; the remaining third were allowed to remain in the vertical position. Following upon stimulation, the horizontally laid roots were decapitated and the tips divided into two halves. These divided root-tips were next placed upon small blocks of gelatin jelly, four being placed on each block. After standing for one hour, so as to allow auxin to diffuse out from the tips into the gelatin, the roots which had been allowed to remain in the vertical position were decapitated and the small blocks of gelatin were eccentrically headed on to the stumps. Half the number of decapitated stumps were reheaded in this way with blocks of gelatin into which auxin from the lower halves of the stimulated tips had diffused (A), and the remaining half were reheaded with blocks of gelatin into which auxin from the upper half tips had diffused (B). The response and average curvature of the stumps after this treatment is given below:

	Number of Seedlings used	*Percentage Response*	*Average Curvature*
(A) Gelatin containing auxin from *lower* halves of tips ..	24	91·6	30·1
(B) Gelatin containing auxin from *upper* halves of tips ..	24	66·6	10·9

It will be seen from these figures that auxin has accumulated to a greater extent on the lower side of the stimulated tip. The curvature shown was in all cases towards the block of gelatin, which is additional evidence that auxin in some unknown way retards the growth of roots.

These experiments of Hawker have interesting implications with regard to the statolith theory of geotropism. The geotropic response shown by *V. Faba*, as well as by *Zea Mays* in Cholodny's investigations and those of Keeble, Nelson and Snow, is brought about by auxin diffusing out equally from all sides of an un-

* *New Phyt.*, 1932, **31**, 321.

stimulated root-tip and becoming redistributed in the stimulated stump. Thus, perception of the stimulus and response to it can take place without any displacement of statolith starch-grains in the root-cap. In any case, as far as *Z. Mays* is concerned, there is no starch in the growing region of the root, so that the geotropic sensitivity of the roots of this plant which has been demonstrated by Keeble and his co-workers, as well as Cholodny, must be due to some other perception mechanism than movable starch-grains.

TWINING PLANTS

It will be convenient here to consider the movements shown by twining plants which do not hold to their supports by special structures such as tendrils (see section on Haptotropism).

The stems of twiners are weak and slender, and they are unable to grow vertically unless they reach some suitable support. In all twiners the growing apex moves about the axis of the older portion of the stem, and in this way describes a more or less circular path. The direction of movement can be either clockwise or anti-clockwise. In the greater majority of climbers the direction of rotatory movement is in the anti-clockwise direction. The hop is an exception to this rule, and so is the honeysuckle, and alternating left and right revolutions have been observed in *Loasa lateritia* and *Bowiea volubilis*. The actual region of rotatory movement is confined usually to the uppermost two or three internodes. This circular motion of the apical region is continued until it comes into contact with some solid support, when it begins to wrap itself round the support, provided of course that the support happens to be of suitable size and shape.

Twiners exhibit a special form of geotropism, known as "lateral geotropism." The young shoots are at first strongly orthotropic and hold themselves erect. At a later stage, when a certain height has been attained, the tip of the shoot bends over, and this bending is not due merely to the weight of the organ, but to active movement, and the shoot tip takes up a practically horizontal position. A rotatory movement now sets in and the horizontally placed shoot-tip revolves round the fixed vertical basal region of the plant. This rotatory motion of the shoot-tip is continued so long as the shoot itself is capable of active growth, and as has already been mentioned, is, as a general rule, in one definite direction, either clockwise or anti-clockwise.

It was at one time considered that the rotatory motions exhibited by the shoot-tips of twiners were purely autonomous in nature. Actually, however, the revolutions are conditioned by gravity alone. In this particular instance we have to deal with increased growth on the sides or flanks of the organ, and not with growth on the upper or lower surfaces. The erect region of the shoot exhibits ordinary negative geotropism, whereas the horizontal portion is diageotropic. Thus, when the right flank of the bent portion is induced to grow more quickly by lateral gravitational stimulus, the horizontal region begins to rotate. To avoid torsion in the basal region of the shoot, it must perform a twist on its own axis and in this way turn another surface towards the right flank. In this manner new surfaces are directed to the right and successively submitted to lateral gravitational stimulation. The spirals are not closely applied to the support at the first, but later each spiral elongates and becomes narrower, and in this way the twiner is able to bind itself more firmly to the supporting object.

Among some twining plants hairs are present upon the stem which act in a subsidiary capacity in holding the stem more firmly to its support. In a few twiners a more complicated apparatus is present to aid in the subsidiary support of the stem. In Strychnos and Uncaria, for example, special hooks are to be found upon the stem; these, however, are unable to perform any special movements (see under Haptotropism) and merely give secondary aid in the support of the stem.

PHOTOTROPISM (HELIOTROPISM)

The incidence of unilateral illumination upon plant organs brings about a response. When the organ bends towards the source of light it is said to be positively phototropic, while if it bends away it is negatively phototropic. Phototropic responses are common in leaves and these organs tend to assume positions so that they do not shade one another. Most stems are positively phototropic, and as a rule leaves are plagiophototropic. By means of this phototropic response the assimilating organs are brought into the most advantageous position for receiving light. The vast majority of roots are insensitive to light. The roots of *Sinapis alba*, *Lepidium sativum* and *Helianthus annuus* show feeble negative phototropism. The roots of *Hyacinthus orientalis* and *Allium sepa*

are said to be slightly positively phototropic. The phototropic response of some plants depends upon the stage of development. The peduncle of *Lynaria cymbalaria* is positively phototropic when the flower opens, but after fertilization it becomes negatively phototropic, and in this way the ripe capsule is pressed into rock crannies or crevices in a wall wherever the plant may happen to be growing.

The phototropic reaction of the leaf is complex. Its final position is in many cases attained by torsions, and the necessary changes in the growth rate which bring about these torsions are often very intricate in nature. Further, in many leaves the change in position due to the stimulus of unilateral illumination is often brought about by a combination of reactions in the base of the blade, in the petiole, and occasionally in the shoot itself. As has already been stated above, the final position taken up by the leaf is at an angle to the incident light, i.e. it is plagiophototropic. Although the leaf reacts in response to light, gravity also plays a part, so that some leaves, at least, take up a horizontal position in the absence of light.

Many of the fruit bodies of the fungi are sensitive to light, and by means of this reaction are able to adjust themselves in a favourable position for the discharge of their spores, for the direction of the light would indicate the direction of an open space.

The sporangiophores of many of the Mucorales show positive phototropism, e.g. *Phycomyces nitens*, *Mucor Mucedo*, and Pilobolus. In Pilobolus the sporangium is hemispherical in shape and borne on an aseptate sporangiophore, which develops a bulbous swelling just beneath the sporangium and another at its base. Fresh sporangia mature daily and are discharged either in the morning or late afternoon. It has been found that the young sporangiophores show positive phototropism. During the early stages of development of the sporangium, curvature is arrested, but later, curvature occurs just below the bulb. In this way a certain accuracy of aim is obtained, for both the terminal sporangium and the bulb are pointed in the direction of the light.

In many Pyrenomycetes among the Ascomycetes, the neck of the perithecium is positively phototropic. In some the reaction is very delicate, and if the direction of the light is frequently changed a zig-zag development of the neck takes place. The asci of *Ascobolus immersus* and also *Ascobolus furfuracens* are positively

phototropic, so that their large spore mass can be ejected in a suitable direction.

Phototropic curvature in the stipe of many Coprini was first recorded by Brefeld as long ago as 1877. Buller found well-marked positive phototropism in the young fruit bodies of *Coprinus niveus* and *Coprinus curtus*. The stipe here ceases to show phototropic response when the pileus begins to expand and now a negatively geotropic reaction is developed. In this way the apex of the stipe is brought out into the light and the horizontal expansion of the pileus is assured.

The etiolated coleoptiles of grasses have been much used for an analysis of phototropic reactions since the pioneer work of

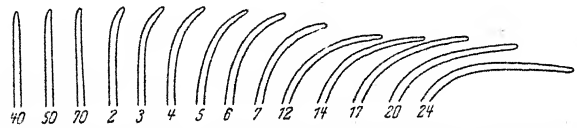


FIG. 43.—Successive positions assumed by coleoptile of oat (Avena) in the dark at 17.5° C., after receiving light for four seconds from a lamp of 30 metre-candles placed at the right-hand side. The numbers show the time after stimulation, the first three in minutes, the rest in hours. (After Arisz.)

Charles Darwin. It was first shown by Darwin that in the coleoptiles of *Avena sativa*, *Phalaris canariensis*, *Panicum miliaceum* and *Setaria viridis* the region of perception and the region of response are not the same. In these coleoptiles when the tip is illuminated, curvature occurs at the base. These observations of Darwin were fully confirmed by Rothert, who showed that the stimulus travels from the apical portion of the coleoptile to the base, which is itself quite insensitive to unilateral illumination. Rothert found that in *Avena sativa* it is the extreme apex of the coleoptile that is especially sensitive, comprising a zone of 1 to 1·5 mm. in length. Beyond this region the coleoptile shows less and less sensitivity to phototropic stimulus. The successive positions assumed by the coleoptile of *A. sativa* in the dark at a temperature of 17·5° C., after receiving light for four seconds from a lamp of 30 metre-candles, is shown in Fig. 43. The source of illumination was on the right of the coleoptiles and after illumination the coleoptiles were rotated on a horizontal klinostat.

It is clear that in the coleoptiles of grasses there is a perceptive region and a motor region. Haberlandt has suggested that the perception of the light stimulus by leaves is as follows: He had

observed that in many cases the epidermal cells of the lamina of the leaf, by virtue of the structure or curvature of their outer walls, act like concentrating lenses, so that if they be brightly illuminated from above, a spot of light is thrown on the back wall of the cell. "The most widely distributed, and at the same time the most perfect type of light-perceiving sensory epithelium, is represented by those forms of adaxial foliar epidermis, in which the outer walls are more or less papillose, while the inner walls are approximately flat and placed parallel to the leaf-surface. . . . For every papillose epidermal cell acts as a plano-convex or condensing lens. The rays falling upon the outer convex wall in the direction parallel to the optical axis of the lens, are refracted so as to converge upon the middle of the inner wall; hence, this central region is brightly lighted, whereas a marginal zone of varying width is not directly illuminated at all, but merely receives a small amount of reflected light from the mesophyll." (Haberlandt.)

This type of lens action is certainly very perfect and photographic images of external objects have been obtained with these structures. Haberlandt considered that the leaf is only in equilibrium when the spot of light falls upon the centre of the cell. If the direction of the light is oblique, then the leaf moves until the light falls normally to the surface and the spot of light is once more brought to the centre of the cell, so that equilibrium is once more restored.

It is, however, extremely doubtful if this theory is correct. It has been found that if the epidermis of leaves be shaved off with a razor, phototropic response is still shown. Again, if the surface of the leaves be covered with paraffin oil, which converts the cells into dispersing lenses, phototropic response is exhibited.

THE MECHANISM OF PHOTOTROPIC CURVATURE

In 1832 it was suggested by de Candolle that phototropic curvature in plant organs was the result of the partial etiolation of the shaded region. The partial etiolation of the shaded region led to greater growth on that side and a curvature towards the source of light occurred. This explanation, however, had the disadvantage that it did not explain the negative phototropism shown by certain organs, for example, roots of *Sinapis alba*, *Helianthus annuus*, the aerial roots of ivy and branched tendrils of Virginian creeper

and the vine. Later, it was suggested by Wolkoff that negative phototropism could also be explained on de Candolle's hypothesis by making the assumption that there was refraction and concentration of the incident light rays upon the tissues away from the source of light, i.e. on the shaded side. In this way the shaded side would be more strongly illuminated than the one facing the source of light, and the growth of these tissues would be retarded and a negative phototropic curvature would result. This suggestion was scouted by Pfeffer, who states: "This quaint idea is totally incorrect."

The "light-growth" response of the sporangiophores of *Phycomyces nitens* and other plant organs discovered by Blaauw has already been described in Chapter XV. Blaauw extended his investigations to the phototropic responses of these organs. The plant to be examined was grown in the dark and then unilaterally illuminated with a definite quantity of light (measured as before in metre-candle-seconds) and then allowed to remain in the dark again. It was found as a result of this unilateral illumination, phototropic curvature occurred, and that the magnitude of the response depended upon the amount of light received. Thus, it did not matter whether the quantity of light received was a low intensity over a long period of time, or a high intensity for a short period of time, provided that the same quantity of light was used, the magnitude of the reaction was the same. It was also found by Blaauw that different quantities of light produced different degrees of curvature in the organ. For example, in the sporangiophore of *Phycomyces nitens*, the reaction could be positive or negative depending on the quantity of light used in the single illumination. As the quantity of light was increased, the response of the sporangiophore altered from a positive reaction to a negative one, and then a second positive curvature occurred.

Blaauw's hypothesis to explain phototropic curvature was really a return to de Candolle's views. For stems, such as the hypocotyl of a seedling of *Helianthus annuus*, he considered that the positive phototropic response shown by this organ is due to a negative growth reaction towards light. It will be recalled that the main objection to de Candolle's hypothesis was the fact that it did not explain negative curvature. Blaauw was able to meet this objection from his investigation of light-growth responses of roots. The roots of the radish, for example, show no light-growth reaction and no phototropic response to unilateral illumination. On the

other hand, certain roots such as *Sinapis alba* show negative curvature to one-sided illumination and also show a small light-growth reaction. The difficulty presented by the positive phototropic curvature of the sporangiophore of Phycomyces, which shows an acceleration of growth after illumination, was explained by Blaauw on the grounds that the colourless sporangiophore acted like a lens and the light was brought to a focus on the opposite side from the source so that this side was more strongly illuminated than the one facing the light and a positive curvature was brought about. Ordinarily it would be expected that, since light brings about an acceleration of growth in the sporangiophore of this plant, there would be an acceleration of growth of the structure facing the source of light and a negative curvature would occur. Thus, Blaauw's explanation is a resuscitation of the views put forward many years before by Wolkoff, that the incident rays are refracted and brought to a focus on the side away from the source of unilateral illumination, leading to an acceleration of growth of this side and so to a positive curvature. Evidence in support of this view was found by trying the effect of unilateral illumination on the sporangiophore of Phycomyces when it is immersed in paraffin oil, which cuts out this lens-action. It was now found that the sporangiophore exhibited negative phototropism.

On Blaauw's view of the phototropic reaction of an organ, light has a direct differential effect on the enlarging region of the sensitive organ, so that the phototropic response of an organ is the secondary phenomenon which must follow as a consequence upon the primary light-growth reaction. Difficulties, however, are encountered when this theory is applied to the phototropic response of the coleoptiles of grasses. In this case the region of curvature is not the region of perception and recent investigations have tended to confirm the earlier views of Boysen Jensen* and others that phototropic response in this case is controlled by hormones.

The coleoptile of a grass, such as that of *Avena sativa*, is a cylindrical structure in which there are two lateral veins. The morphological nature of the coleoptile has given rise to a great deal of discussion as to whether it is to be regarded as a cotyledon or not. To those interested in a discussion of this kind, the monograph by Agnes Arber, *Monocotyledons*, is recommended. What-

* *Ber. deut. bot. Ges.*, 1910, **28**, 118; 1913, **31**, 559.

ever may be the true morphological nature of this structure, in its response to unilateral illumination it shows that the perceptive and responsive regions are separate.

Rothert, who repeated the investigations of Charles Darwin on the phototropic responses of the coleoptiles of grasses, found that when these lateral veins were cut the stimulus was still able to pass and curvature was shown to one-sided illumination. Rothert's work was in turn repeated by Fitting,* who obtained the same result. The very careful investigations of Boysen Jensen gave the first definite indication that some chemical substance was involved and that this substance was generated at the tip and then travelled down to the motor region at the base of the coleoptile.

In the first place it was found by Boysen Jensen that if the vein facing the source of light were cut, the coleoptile still responded and showed positive curvature. On the other hand, if the vein on the side away from the source of illumination were cut, there was no reaction. Boysen Jensen worked under the ordinary humidity conditions prevailing in the laboratory, but he found that if the vein on the side away from the source of light were cut when the coleoptiles were maintained in a saturated atmosphere, positive curvature still occurred; a result in full agreement with the previous investigations of Rothert and Fitting. He also obtained positive curvature if the vein were cut under water.

From these experimental observations it was concluded by Boysen Jensen that if a chemical substance or substances was responsible for the curvature of the coleoptile in response to unilateral light, it must pass down to the motor region through the vein away from the source of light. Further, the fact that a response was shown when the coleoptile was kept in a saturated atmosphere shows that it can pass through a water-filled gap.

Further evidence in support of the view that some chemical entity is involved in the phototropic response of the coleoptile was obtained by Boysen Jensen by decapitating a coleoptile and replacing the tip with a gelatin base between it and the stump. On illumination a positive response was obtained. It is clear from this experiment that the stimulus can pass across a gelatin film.

An extension of Boysen Jensen's work was carried out by Paal† using the coleoptile of the grass *Coix lacrima*. He first of all

* *Jahrb. f. wiss. Bot.*, 1907, **44**, 177. † *Jahrb. f. wiss. Bot.*, 1919, **58**, 406.

showed that none of the results obtained are due to stray light from the illuminated apex. He also found that if a coleoptile were decapitated and the tip replaced on the stump eccentrically, a curvature of the coleoptile away from the side covered by the tip took place. It was considered that a growth-accelerating substance was formed in the tip, which passed across the moist discontinuity between tip and stump and down the side of the stump covered by the tip. As a result, acceleration of growth of this side of the coleoptile was brought about and a curvature occurred.

It has been shown by Stark* that it does not matter whether the tips of foreign genera are placed on the stumps, a positive response being exhibited. In one particular example he placed the tip from a coleoptile of Avena on the stump of a coleoptile of a different genus, Hordeum. On illumination a response was still given. In this particular case, Avena tip on Hordeum stump, the response was very much stronger than when a Hordeum tip was replaced on its own stump.

The phototropic response of the grass coleoptile is best explained on Pfeffer's view, namely, that the action of light is to act as a releasing stimulus, and that there is no quantitative relationship between the externally supplied energy and the resultant response of the plant.

It has now been proved beyond reasonable doubt that a hormone (auxin, see Chapter XV) is responsible for bringing about the phototropic curvature of the coleoptile. F. W. Went† and others working at Utrecht have carried out a particularly brilliant series of investigations on this question. It was established by Went that the tip of the Avena coleoptile is rich in auxin, whereas the remaining part of the structure is poor in auxin. Now it is the tip of the coleoptile that is the perceptive region; and Went proceeded to examine the distribution of auxin in the apex under the influence of unilateral illumination. In the first place the tips were unilaterally illuminated with 1,000 metre-candle-seconds of light, and the coleoptiles were then decapitated and the tips placed on standard agar blocks with a safety razor blade between the sides that had been towards the source of light and also on the sides that had been facing away from the light. These half-blocks of agar were then placed eccentrically on the stumps of a different set of coleoptiles in the dark. The half-blocks of

* *Jahrb. f. wiss. Bot.*, 1921, **60**, 67. † *Rec. Trav. bot. Néerl.*, 1928, **25**, 1.

agar could not be replaced on the original stumps, because it was found that the decapitated stump becomes sensitive to light two hours after it has been decapitated, owing to the regeneration of a new "physiological tip," i.e. to the regeneration of fresh auxin. When the half-agar blocks were placed eccentrically on the stumps, growth-curvatures occurred and the angle of divergence was measured. At 25° C. curvature took place in 110 to 120 minutes, and the coleoptiles bent away from the side on which the block of agar was placed. After 170 minutes or so, curvature in the opposite direction occurred. This second curvature was considered by Went to be due to the regeneration of a new physiological tip which is apparently first formed on the side of the decapitated stump away from the block of jelly, and from here a fresh supply of auxin is released into the base of the coleoptile.

The average of several experiments showed that there was either destruction or annulment of 16 per cent of the auxin in entire tips by unilateral illumination of 1,000 m.-c.-s. Further examination showed that of the remaining 84 per cent of auxin, 57 per cent was present in the half-tip from the side which had not been illuminated and 27 per cent in the half-tip which had been illuminated. In the controls, tips of coleoptiles which had been kept in darkness, the auxin was uniformly distributed. From these experimental results it was considered by Went that unilateral illumination of the coleoptile tips leads to a redistribution of auxin. The auxin moves laterally to the side away from the source of illumination, and is then transported downwards to the enlarging cells at the base of the coleoptile on this side. As a result of the accelerating influence upon the enlargement of these cells a positive curvature results. On Went's view of phototropic response, curvature is the result of the redistribution or polarization of auxin.

There has been a good deal of controversy over the rival theories of Blaauw and Went as to the cause of phototropic curvature. The strongest argument advanced by Blaauw in favour of his theory of the direct action of light bringing about curvature is that such roots as do show a light-growth reaction are also sensitive to unilateral illumination and show a phototropic reaction, whereas roots which are not phototropic show no light-growth reaction.

These rival views have been more or less brought into harmony

by van Overbeek.* He showed that in the hypocotyl of *Raphanus sativus* submitted to unilateral illumination, auxin tends to pass to the side away from the source of illumination. Agar blocks containing auxin were placed eccentrically on a portion of the detached hypocotyl, and two agar blocks containing no auxin were placed at the base and the auxin from the block of agar placed eccentrically at the upper end of the cylinder could in this way be collected separately. It would be expected on *a priori* grounds that the greater amount of auxin would collect in the block at the base of the cylinder immediately under the block placed on the summit. This was only found to happen if the experiment were carried out in the dark, or if the cylinder of hypocotyl were equally illuminated on all sides. If it were unilaterally illuminated, auxin was always found on the side away from the source of illumination, no matter if the block of agar containing auxin were facing the light. Thus under conditions of unilateral illumination auxin passes to the shaded side, so that translocation is in an oblique direction to the opposite side and not longitudinal as in the dark, or if the cylinder be equally these illuminated all round.

A further effect of light on the hypocotyl of Raphanus was discovered by van Overbeek. He found that light had a direct effect upon the cells of the hypocotyl. Thus it did not matter whether the hypocotyl were illuminated with unilateral light or whether there were equality of illumination on all sides, the effect of the light was to make the cells less sensitive to auxin. The same concentration of auxin was found to bring about a greater elongation of the cells in the dark than in the light. It therefore becomes possible to combine the rival theories of Blaauw and Went. In unilateral light auxin is transported obliquely to the side away from the source of illumination and at the same time there is a greater diminution in the sensitivity of the cells of the hypocotyl to the action of the hormone on the side facing the light than in those on the shaded side. As a result of the combined influence of these two actions curvature must take place.

Phototaxis.—The response of motile organisms, such as certain free-swimming unicellular plants, zoospores, etc., to unilateral light is termed *phototaxis.* If the organism moves towards the source of light it is said to be positively phototactic, and if it moves away negatively phototactic. A number of zoospores of both algae and

* *Rev. Trav. Bot. Néerl.*, 1933, **30**, 537.

fungi are sensitive to unidirectional light. The long axis of the zoospore becomes placed parallel to the direction of the light, and it swims in a definite direction towards the source of illumination. In light of moderate intensity, the zoospore turns the anterior end towards the light, whereas if the light intensity be too strong the anterior end is turned away, and the direction of movement of the motile spore follows in the same direction as the anterior end.

The zoospores of algae show a considerable amount of variability in their response to unilateral light. The zoospores of Vaucheria and the small yellow zoospores of *Bryopsis plumosa* are said to be insensitive to unidirectional light. On the other hand, the large green zoospores of *B. plumosa* show a positive reaction. The zoospores of *Codium tomentosum* as well as those of *Ectocarpus firmus* only show a very small phototactic response, whereas the spermatozoids of Fucus are strongly phototactic.

Among the fungi, a phototactic reaction has been observed in the zoospores of *Chytridium vorax* and *Polyphagus Euglenae*. Both these species are active parasites, the first on *Chlamydococcus pluvialis* and the second on *Euglena viridis*. The host plants are also phototactic, since they obtain their supplies of carbon by photosynthesis, and the phototactic reaction of the parasites brings them into the region where the host plants are to be found.

CHEMOTROPISM

Chemical substances are able to bring about orienting tropic movements in plants. Plant reactions to chemical stimuli have been most studied for freely motile organisms (chemotaxis), but there is a considerable amount of information available with regard to the tropic curvatures of pollen-tubes and fungal hyphae under chemotropic stimulation.

When the plant organ moves towards the source of the diffusing chemical substance, it is termed positive chemotropism, when it grows away in the opposite direction it is negative chemotropism. The response of plants to different chemotropic stimuli varies widely. The chemical nature of the stimulating substance is also important and compounds of similar constitution may exert very dissimilar physiological reactions.

Chemotropic stimuli appear to play an important part in directing the pollen-tube to the ovule. It has been shown by

Lidforss that when the pollen grains of *Vallota purpurea* are grown in artificial culture on a sugar medium containing diastase made up in gelatin and are placed in the centre of the plate, the developing pollen-tubes show positive chemotropism and the tubes are directed towards the mass of diastase. In the same way, pollen grains allowed to germinate on nutrient jelly in which pieces of the pistil have been placed grow away from the air and towards the portions of pistil. In this case the pollen-tubes have shown positive chemotropism towards the pieces of pistil.

In the fungi the most marked chemotropic response of the developing hyphae and germ-tubes is a negative reaction, i.e. they show a tendency to grow away from their own metabolic products. It is sometimes said that fungal hyphae tend to grow away from their own "staling" products.

It was shown by Miyoshi that fungal hyphae exhibit negative chemotropism towards acids, alkalis, alcohol, and even certain neutral salts, so that the products of metabolism are not the only factors which are able to bring about a negative chemotropic response in the fungi. He also advanced evidence that the hyphae show positive chemotropism towards sugar and various nutrient substances. His data on this aspect of the matter, however, are vitiated by the fact that he was not aware of the negative chemotropic response of the hyphae towards their own staling products. Thus, although a positive response was obtained and the hyphae grew towards the fresh medium, curvature was really dependent on the repellent influence of the old.

Clark,* who investigated the chemotropic response of *Rhizopus nigricans*, found that the hyphae exhibit negative chemotropism to some secretion of their own mycelium. In the same way Fulton,† who examined the growth of germ-tubes of several different species of fungi, found that they tend to grow away from a region in which the same kind of hyphae are present to a region free of hyphae, or in which the hyphae are less abundant. This marked negative chemotropic response of fungal hyphae is well shown in the manner of their growth, either in artificial culture or growing under natural conditions. The hyphae tend to grow equally in all directions away from the centre of infection. Many fungal colonies are characterized by alternate zones of dense and sparse growth, and it has been suggested by Stevens and Hall‡ that this type of growth results from the deposition of

* *Bot. Gaz.*, 1902, **33**, 24. † Ibid., 1906, **41**, 81. ‡ Ibid., 1909, **48**, 1.

the products of katabolism and in this region growth will be inhibited to a certain extent until a few scattered hyphae have succeeded in passing beyond this inhibiting region, when they are able to give rise to a fresh region of luxuriant growth.

It has been shown by Balls* for the so-called sore-skin fungus of cotton, that when the nutrient medium is the limiting factor for growth, high temperatures bring growth to a standstill more rapidly than low. This result is apparently due to the fact that at high temperatures growth is accelerated and there is a more rapid accumulation of staling products, and is not to be attributed to the earlier exhaustion of the food supply. When the medium at this stage was diluted with an equal quantity of water, it was found to be capable of supporting further growth. By dilution of the medium the concentration of staling products present was sufficiently reduced to prevent their inhibiting action.

The chemotropic responses of fungal hyphae have been fully investigated by Graves† for *Rhizopus nigricans*. Germ-tubes of this form were grown on contrasted agar media which were separated by a perforated mica sheet. A turnip juice nutrient medium was employed and in the first place it was found that developing hyphae grew down towards the perforations to fresh but identical agar medium on the other side of the mica sheet. When an agar medium was placed on the lower side of the mica plate in which growth had already taken place, the hyphae turned away towards fresh agar in which no growth had occurred. Even when only plain agar without any nutrient was placed on the lower side of the mica sheet, the hyphae grew down towards it. The repellent influence of the staling products of the fungus was found to be destroyed by heating to a temperature of 100° C.

Although the most marked chemotropic response of fungal hyphae is a negative one, it was shown by Graves that they also show a definite but weak positive response. It was found by him that glucose and sucrose exert a small positive chemotropic influence on *R. nigricans*.

Since the dominant response of the hyphae of saprophytic fungi is a negative one towards their own staling products, and this is the main factor governing the distribution of these forms in culture, it may reasonably be inferred that negative chemotropism towards the products of metabolism also governs the distribution of parasitic forms in the host plant. That this is the case has been

* *Ann. Bot.*, 1908, **22**, 558.

† *Bot. Gaz.*, 1916, **62**, 337.

shown by W. Robinson* for the germ-tubes of *Puccinia malvacearum*. He found that the germ-tubes formed from the basidiospores do not show positive chemotropism towards pieces of the leaf of the host plant.

Chemotropic stimuli apparently also play a part in directing the antheridial hyphae of Saprolegnia into contact with the oogonium. They may also play a part in directing the growth of the conjugating tubes of Spirogyra and other Conjugatae into coming into contact so that fertilization can take place.

Chemotaxis.—A number of investigations on the chemotactic responses of freely motile organisms was carried out by Pfeffer. His method was to fill small capillary tubes open at one end with the compound, the effect of which was to be investigated, and to place this tube in a drop of water containing the motile organisms. If the substance exerted a positive chemotactic effect the organisms were found to collect round the mouth of the tube, whereas if it exerted a negative influence, they collected some distance away from the mouth. Pfeffer showed that the spermatozoids of ferns show a strong positive chemotactic response to a 0·01 per cent of sodium malate, whereas a solution of 0·001 per cent strength only exerts a feeble response. Malic acid itself also gave rise to a strong positive response, and it was found that it did not matter whether the optically active isomerides of the acid were used or the inactive form, the same positive response being exhibited. For the spermatozoids of Mosses, the stimulating substance was found to be sucrose. Even as low a concentration as 0·001 per cent gave rise to a perceptible response.

HYDROTROPISM

Tropic curvatures by plant organs towards water are shown by many plants. Main and lateral roots show positive hydrotropism and tend to grow towards moister soil or other substratum. Positive hydrotropism may be of importance to some plants; for example, those inhabiting the sides of cliffs. The roots would either curve back toward the surface of the cliff or keep buried in the soil. The rhizoids of Marchantia are positively hydrotropic and the suggestion has been made that this fact is of importance in anchoring this plant when it is growing on the sides of a rocky substratum.

Hydrotropism of roots can be demonstrated by allowing seeds

* *Ann. Bot.*, 1914, **28**, 331.

to germinate in a sieve filled with sawdust and suspended in such a way that the lower end is at an angle of 45 degrees from the horizontal. The primary roots of the germinating seeds will break through the perforations in the sieve, but instead of growing vertically downwards, they bend laterally towards the bottom of the sieve and then proceed to grow in a downward direction along the outer surface, to which they become closely pressed.

Positive hydrotropism has been described in the hyphae of *Mucor Mucedo* by Fulton,* who found that the hyphae grew through the perforation in a mica plate from gelatin jelly into water. *Rhizopus nigricans*, under the same conditions, grew into the relatively moist gelatin near the perforations, but turned away from the fluid water beneath.

On the other hand, the sporangiophores of various Mucorales have been described as being negatively hydrotropic.

Hydrotaxis.—The plasmodium of myxomycetes show positive hydrotaxis and creep towards a moist substratum. At the advent of the fruiting stage, negative hydrotaxis is said to occur and the plasmodium creeps on to the surface of the moist substratum and then up the sporangial stalks out of the moist region.

HAPTOTROPISM (THIGMOTROPISM)

This term is given to the reaction of non-twining climbers, such as tendril-plants or plants in which different members, such as the leaf-tip of *Gloriosa superba*, are sensitive to contact stimulus.

In the tendril-climbers, such as *Bryonia dioica*, *Cobaea scandens*, *Lathyrus odoratus* and *Pisum sativum*, the tendrils are sensitive to contact. When a tendril comes into contact with a solid object, the process of coiling begins near the tip. The tendril now wraps itself round the support, the coiling movement resulting from unequal growth on the two opposite sides following upon the stimulus of contact. The region of the tendril between the point of contact and the base of the organ also becomes coiled in the shape of a spiral spring, so that the plant is drawn nearer its support. The tendril can only become attached to its support while it is actively growing, and if a tendril does not meet a suitable support during its growth period it generally withers and dies. The possibility of breakage when a tendril-climber is shaken is largely overcome by the fact that the spiral coiling of the region

* *Bot. Gaz.*, 1906, **41**, 81.

between the support and the stem acts like a spring against external shocks such as wind and rain.

The morphological nature of tendrils varies greatly in different plants. In some, the whole leaf develops into a tendril (*Lathyrus aphaca*), while in *Cobaea scandens* it is only a portion of the compound leaf that develops into a tendril. In some plants it is the petiole that is sensitive to contact stimulus, e.g. *Clematis vitalba* and *Solanum jasminoides*. In the orchid Vanilla, the aerial roots are feebly sensitive to contact stimulus and function as root tendrils.

It was first observed by Charles Darwin that tendrils are only sensitive when they come into contact with solid objects. Drops of water do not stimulate them. Anatomically tendrils show certain features which are considered to facilitate the reception of contact stimuli. In this connection various members of the Curcubitaceae have been especially investigated. Pfeffer has shown that in the members of this family there are special "tactile pits," which are unthickened areas, very similar to ordinary pits in the cell wall. In the thick external epidermal walls of the tendrils of these plants, these minute pits extend the cell cavity outwards almost to the outer surface of the wall which is very thin at these points. The thinness of the cell wall at these places is supposed to reduce to a minimum the energy necessary to deform the cellulose envelope, and in this way the larger proportion of the total energy of the stimulus is employed in deforming the sensitive protoplasm.

The behaviour of the parasite Cuscuta is in many respects peculiar. This plant behaves in some ways like an ordinary twiner and in some ways like a tendril-plant. It has been shown by Peirce that Cuscuta exhibits two conditions which alternate in a regular manner. In the first condition it twines round the support like an ordinary twiner, in a left-hand manner. The apex of the shoot performs a number of steep spirals round the vertical support. Following upon this phase, comes the second stage of reaction in which Cuscuta behaves like a tendril-plant. The spirals round the support become less steep and the coils grasp it very much more firmly. In this second coiling phase the plant is sensitive to contact stimulus with solid objects, but not to moist gelatin. Thus the sensitivity of the stem during the second phase of development is comparable with the condition exhibited by tendrils. It should also be noted that unlike tendrils, Cuscuta during the haptotropic stage of sensitivity is also geotropically

sensitive and will only twist itself round a vertical support. Further, the haustoria which are forced into the host plant by this parasite are induced by haptotropic stimulus.

Haptotropic phenomena are also exhibited by certain of the so-called "carnivorous" plants. The case of the sun-dew, *Drosera rotundifolia*, was investigated by Charles Darwin, who showed that the tentacles which are present on the almost circular laminae of the leaves are sensitive to contact stimulus. The tentacles which are present in the middle of the leaf-blade are glandular, possess short stalks and stand erect, whereas the tentacles situated near the periphery of the blade possess long stalks and are bent in an outward direction. A sticky, acid secretion containing some proteolytic enzyme or enzymes is formed at the tip of each tentacle and any insect which may happen to alight upon it is held fast and cannot escape and is eventually digested. The moment an insect alights upon a central tentacle, the peripheral tentacles are stimulated into action and curve towards the trapped insect and come into contact with it. If the insect should happen to come into contact with one of the central tentacles which have short stalks, these still remain erect, but in some way the stimulus is passed out from the centre of the leaf to the peripheral long-stalked tentacles and these proceed to curve inwards. On the other hand, should an insect come into contact with one of the peripheral tentacles, this bends over towards the centre and the prey is carried towards the middle of the leaf. Once arrived here, a stimulus is passed to the remaining peripheral tentacles which also begin to curve towards the middle of the leaf-blade. It is evident then that the stimulus can only be transmitted by the central tentacles.

Drosera will respond to both chemical and mechanical stimuli. Its reaction to chemical stimuli such as white of egg or lean meat is very rapid. It was found by Darwin that water and other neutral liquids do not affect the tentacles, even when directed against them with considerable force. It was also found by Pfeffer that a glass rod covered with gelatin did not induce any movement of the tentacles (cf. tendrils). The tentacles, however, can be stimulated by a number of successive blows from a chip of wood or a pencil. In this respect the tentacles behave like tendrils. It is only the glandular region of the tentacle that is sensitive to contact, so that the region of perception is strictly localized, whereas curvature is confined to the base of the stalk.

RHEOTROPISM

The phenomenon of rheotropism was first described in 1883 by Jönsson for seedling roots. It was found that when such roots were grown in running water they exhibited a curvature in a direction away from the direction of flow of the water, i.e. they showed positive rheotropism. The behaviour of the roots of a number of cruciferous plants cultivated in running water was examined by Newcombe.* He found that the minimum rate of flow required to bring about a reaction was 2 cm. per minute, but the greatest reaction was shown when the speed of flow was 100 to 500 cm. per minute. When the speed of flow was 1,000 cm. per minute, negative rheotropism was exhibited, i.e. curvature occurred in the same direction as the flow of water. This result, however, was in all probability due to purely mechanical causes and was not an irritability phenomenon. The roots of *Vicia sativa* and *Zea Mays* also show rheotropism, but a number of plants are insensitive to this form of stimulation.

MOVEMENTS OF VARIATION

We have already considered some of the more important growth movements exhibited by plant organs and whole plants under the influence of external stimuli. It will be recalled that these movements only occur in actively growing organs, although exceptions to this rule were encountered, and further, that these movements cease when growth of the organ is completed. The movements of mature organs, whether induced by external stimuli (paratonic movements) or whether they be due to internal causes (autonomic movements), are known as *movements of variation.* It will also be remembered that when the orientation of a plant member shows a relation to the direction of application of the stimulus, it is termed a tropism, whereas when the stimulus is diffuse, or when the movement of a plant organ is exclusively determined by the inherent properties of the irritable member, whether the impinging stimulus be unilateral or not, it is termed a nastic movement.

AUTONOMIC MOVEMENTS OF VARIATION

Autonomic movements of variation are limited in number. The best known example is perhaps that of *Desmodium gyrans*, the so-

* *Bot. Gaz.*, 1902, **33**, 177.

called "Telegraph plant." The leaves of this plant are tripartite. The terminal leaflet is large and does not perform very noticeable movements, but the lateral pair of leaflets show quite rapid autonomous movements which can be followed with the naked eye. The movement follows an elliptical path and the whole movement occupies between $1\frac{1}{2}$ to 3 minutes. The movement of the leaflets is not smooth and regular, but rather jerky in nature, and more rapid in the downward than in the upward direction. The rate of movement depends upon the temperature. In a high summer temperature the rate of movement of the leaflets is speeded up. Similar movements of leaflets have been described in other plants. Thus the terminal leaflet of the red clover completes slow upward and downward movements. The rate of movement, however, compared with the leaflets of Desmodium is extremely slow. Under summer conditions the rate may be anything between one and four hours for an upward and downward movement to take place.

NYCTINASTIC MOVEMENTS

The leaves and floral organs of many plants take up different positions during the evening hours from that which they occupied during the day. Many flowers unfold their petals or perianth leaves during the day and close them at night. In the case of the Compositae the whole inflorescence, the capitulum, is able in a number of cases to perform this day and night movement. This is known as a *nyctinastic* movement.

The nyctinastic movements of foliage leaves can only take place while the leaf is in a condition of active growth and the amplitude of the movement decreases with the age of the leaf. In certain leaves, however, the power of nyctinastic movement is retained when the leaf has reached maturity. This nyctinastic movement of leaves is to be found in many families, such as the Leguminosae, Oxalidaceae, Euphorbiaceae and Marantaceae among angiosperms, and is also shown by the aquatic fern Marsilia.

Plants which exhibit nyctinastic movements react to variations in the degree of different stimuli, such as temperature, light and so forth. These are nastic movements in contra-distinction to tropic movements. In other words, the stimulus that induces movement is diffuse and not unidirectional.

The petals of such flowers as those of the tulip and crocus when maintained at a constant temperature will open when illuminated and close when darkened. This reaction will only take place before the petals are fully expanded and thus they exhibit *photonasticism*. On the other hand, if the flowers be kept under a constant light intensity, and the temperature be varied, they will open in the higher temperature and close in the colder, i.e. they show *thermonasticism*.

These photo- and thermonastic movements are brought about by growth curvatures. When the lower side of an organ grows more quickly than the upper it is termed *hyponastic* growth, whereas when the upper surface of an organ grows more rapidly than the lower it is described as *epinastic* growth.

SEISMONIC IRRITABILITY

A number of plants are known which respond in a characteristic manner to intense mechanical shock. Such plants are said to show *seismonic* irritability. This seismonic irritability should be distinguished from contact or haptotropic (thigmotropic) sensitivity exhibited by such organs as tendrils. Haptotropic irritability is only shown by an organ when it comes into contact with a solid body, and such agencies as wind, rain, or rubbing with a wet rod covered with a layer of gelatin do not awaken any response. On the other hand, plants which exhibit seismonic irritability respond when struck a blow, or when they come into contact with a solid body, and in some cases they will even react to vibrations propagated through the ground.

These plants perform paratonic nastic movements of variation in response to the stimuli of contact, injury and shock. *Mimosa pudica* is perhaps the most quoted example of a plant which is sensitive to shock, but a number of other cases are also known. The leaves of *Biophytum sensitivum* also react to shock very readily, while a less sensitive species is *Oxalis acetosella* in which the leaves will only show a response after vigorous shaking. The stamens of *Centaurea jacea* and *Cynara scolymus* show seismonic sensitivity and when stimulated by rubbing contract together. Stamens sensitive to shock are also to be found in the Berberidaceae and Cistaceae.

The analysis of seismonic irritability has been most fully investigated in *Mimosa pudica*. The reaction of *M. pudica* to a blow is particularly rapid and the leaves pass from the unstimulated

to the stimulated state very quickly (Fig. 44). When this plant is stimulated the main petiole sinks, the secondary petioles become less spreading and the leaflets fold together in pairs.

The motor region in Mimosa is located in the pulvinus, a more or less flattened structure only able to bend in one plane. The cells composing the lower half of the pulvinus are thin-walled, whereas in the upper portion fairly thick-walled cells are present. The movement performed by the leaf after stimulation is due to changes in the turgor of the cells

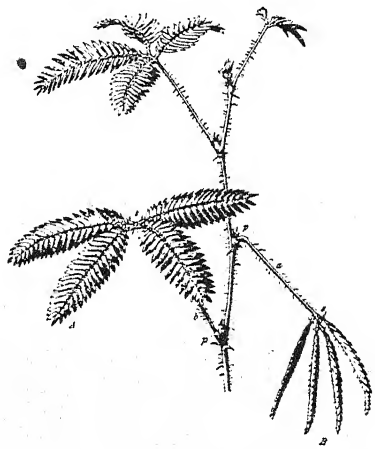


FIG. 44.—Seismonic irritability shown by *Mimosa pudica*.

of the pulvinus. The turgor of the cells situated in the lower half of the pulvinus shows a considerable decrease, as much as 2 to 5 atmospheres. Water escapes out of these cells into the intercellular spaces which previously contained air. The actual downward movement of the petiole is partly brought about by a contraction of the walls of these thin-walled parenchymatous cells following upon the decrease in turgor, but this movement is further accentuated by the expansion of the cells in the upper half of the pulvinus. The foliage leaves, as well as the pulvinus in Mimosa, are provided with small hairs or bristles, and these apparently are also sensitive to touch and the stimulus can be passed to the motor region by stimulation of these structures.

Although, under normal conditions, the propagation of a stimulus in Mimosa is comparatively rapid for a plant, under other conditions only slow and feeble response may be given.

Thus, if the plant be kept at a temperature of 5° C. or 10° C. for some time, it loses tone and will only give a slow response or even no response at all to stimulation. Or again, if the plant be stimulated a number of times it will pass into a state of rigor and show no reaction. This result is due to the motor apparatus being unable temporarily to perceive the stimulus, and not to the motor apparatus being thrown out of action, for while still in this condition of rigor to mechanical shock, Mimosa is still sensitive to other stimuli, such as phototropic or photonastic stimulus. Both high and low temperatures lead to lack of response in Mimosa to seismonic irritability. It has already been seen that if the temperature falls below 15° C., no response is shown and if the temperature be raised to 40° C. for an hour, or higher temperatures still (45° C. and 50° C.) for still shorter periods, transitory heat-rigor is produced. Low partial pressures of oxygen lead to feeble seismonic movement, while anaesthetics such as ether and chloroform, in moderate doses, inhibit all movement.

It was first shown in 1824 by Dutrochet that the stimulus in Mimosa is conducted through the vascular bundles. Later (1873) it was found by Pfeffer that the stimulus is able to pass across chloroformed regions of the stem, while Haberlandt discovered that the stimulus could pass across dead regions of the stem and a similar result was obtained by MacDougal. It was considered by Dutrochet, Sachs, Pfeffer and MacDougal that the stimulus travelled through the xylem and that the transmission was due to the propulsion of water. An alternative view was advanced by Haberlandt, namely, that the stimulus travelled through the phloem. According to this view pressure is exerted upon the turgescent cells of the phloem when the pulvinus of a pinnule moves upwards in response to stimulation. This rise in pressure is propagated along the system of sieve-tubes in the form of a pressure-wave. Such a rise of pressure does not extend to the subpetiole, which is comparatively insensitive, nor does it penetrate as far as the main pulvinus. It remains localized and only extends from one pair of pinnules to the rest. On the other hand, severe injury, such as cutting off a pinnule, would immediately destroy the turgor of the injured transmitting cells. As a result, a large local fall of turgor would occur and this would be propagated through the sieve-tubes as a "wave of relaxation or negative tension." Violent injury of this kind, on Haberlandt's view, would lead to a large initial change in pressure, so that wound or

traumatic stimulus will be transmitted over a relatively larger distance and will not only reach the main pulvinus but pass through the stem too and affect other leaves.

The problem of the passage of the stimulus in Mimosa has been re-investigated within recent times by various workers. Ricca* was able to confirm the older view of Dutrochet, Sachs, Pfeffer and MacDougal that the stimulus is able to pass through the xylem. He found that if a region of the stem were killed by steam or separated by a water-gap, the stimulus was still able to pass in an upward or downward direction. Evidently then the stimulus involved is in the nature of a chemical substance or hormone. The later investigations of Snow† fully confirmed Ricca's observations. He was also able to show that the phloem of the stem is quite insensitive to the passage of the stimulus, but that it is conducted through the phloem in the petiole. This method of conduction in the petiole has been called by Snow "high-speed conduction." In the plant the hormone responsible for this seismonic reaction of Mimosa is thermostable, but watery extracts are thermolabile. Boiling of the watery extracts from the tissues inactivated the hormone.

A cursory examination of the chemical properties of the hormone was made by Snow. He found that it was not precipitated by lead acetate and gave none of the colour reactions of a protein and that it is able to diffuse through a collodion thimble without losing its properties.

The seismonic irritability of Mimosa has also been further investigated by Ball.‡ He, too, was able to confirm the statements of Ricca and Snow that the normal method of conduction of the stimulus in the stem is through the xylem. In this case the stimulus travels at a rate of about 15 to 28 cm. per minute. It was also discovered by Ball that the stimulus can travel through the pith at the very high rate of 200 cm. per minute. This very rapid method of conduction can be shown by completely submerging cut shoots in water. It was proved that the hormone does not travel in the phloem of the stem, because on decortication and complete removal of all tissues external to the wood, the stimulus still travelled at the same rate as before. Under normal conditions the hormone is released at the point of stimula-

* *Nuovo Giov. bot. Ital.*, N.S., 1916, **23**, 51.
† *Proc. Roy. Soc.* (*Lond.*), 1924, **96B**, 349; 1925, **98B**, 188.
‡ *New Phyt.*, 1927, **26**, 148.

tion and travels in the transpiration stream through the xylem. This is the method of translocation when the water tension in the xylem is high and the turgor of the tissues low. When the turgor of the tissues is high, the hormone is still released at the point of stimulation, but instead of passing up the xylem in the transpiration stream, it brings about contraction of the neighbouring cells, possibly of the medulla. These cells in turn release more hormone, and in this manner a highly efficient relay mechanism is set up whereby the hormone can pass in either direction in the plant and is quite independent of the transpiration stream.

Ball was able to show that either method of translocation of the hormone can operate at one and the same time. It is possible that the relay mechanism described for the passage of the hormone through the medullary cells is of the same nature as the "high-speed conduction" described by Snow for the phloem of the petiole.

Houiwink* has also examined the passage of the stimulus in Mimosa by studying the movement of the leaves and the internal fluctuations in electrical potential. If the plant were stimulated without wounding by using drops of water cooled to below 10° C., the stimulus passed through the living cells and was unable to pass through a zone of killed tissue in the petiole, nor could it pass through a zone of petiolar tissue if this were chilled to 5° C. On the other hand, if wounding or burning of the tissues were used to stimulate the plant, the formation of the stimulating substance or hormone brought about a change in potential. The stimulus generated in this way is able to pass through a zone of dead tissue and is transported in the transpiration stream. It is evident that the stimulus in Mimosa can travel in a number of different ways depending upon the conditions of stimulation. Houiwink recognizes three methods of conduction: (1) by the action of living cells when only a small shock is applied, (2) transport of a wound substance in the xylem and (3) some special rapid method of conduction which is localized to the main pulvinus.

* *Rev. Trav. Bot. Néerl.*, 1935, **32**, 51.

APPENDIX

THE CONCEPTION OF pH

THE degree of acidity or alkalinity of the medium markedly affects biochemical reactions. The precise degree of acidity or alkalinity of a solution has a definite relation to the number of hydrogen ions that are present in such a solution. In neutral water the number of hydrogen ions is very small (1 gm. in 10 million litres of water), while in a biological fluid such as arterial blood the amount is yet smaller. It is obvious that the significance of such a hydrogen concentration as that of water is very difficult to visualize, and to overcome this difficulty the conception of pH has been introduced.

The definition given to a normal solution of an acid or salt is that 1 gm. of hydrogen or its equivalent is dissolved in 1 litre of water. In the case of monobasic acids, such as nitric, hydrochloric or acetic, a normal solution would contain the molecular weight in grams per litre. Thus a normal solution of nitric acid would contain 63 gm. per litre, and the number of grams present per litre in normal solutions of hydrochloric acid and acetic acid would be 36·5 gm. and 60 gm. respectively. Normal solutions of dibasic acids, such as sulphuric and oxalic, would have half the molecular weight in grams dissolved in a litre of water, and with tribasic acids, such as phosphoric acid, one-third the molecular weight in grams dissolved in a litre of water would represent a normal solution.

Each of these normal solutions contains 1 gm. of hydrogen per litre, but although they have the same amount of hydrogen this element is not ionized to the same extent in each case. The test of a strong or a weak acid is whether it dissociates strongly or weakly into hydrogen ions in solution. Thus hydrochloric and nitric acid are said to be strong acids, while acetic, oxalic and tartaric acids are said to be weak acids. Hydrochloric and nitric acid dissociate in solution to a much greater extent than say acetic or oxalic acid. Yet a normal solution of a weak acid contains 1 gm. of hydrogen per litre and requires precisely the same amount of a normal solution of an alkali to neutralize it completely as a strong one. Thus a normal solution of hydrochloric acid requires 40 gm. of sodium hydroxide to neutralize

it and a normal solution of acetic acid requires the same amount of alkali. The difference in strength between hydrochloric acid on the one hand and acetic acid on the other, lies in the fact that in dilute solution hydrochloric acid is practically entirely dissociated into hydrogen ions, whereas acetic acid is dissociated to a very much smaller extent. In a 0·001N solution of hydrochloric acid approximately 97 per cent of the hydrogen is ionized, while in a 0·001N solution of acetic acid only 13·6 per cent of the hydrogen is dissociated in this way. The fact that a weak acid which is only dissociated into hydrogen ions to a small extent requires exactly the same amount of alkali as a strong acid of the same normality for neutralization, is due to the fact that as the hydrogen ions in a weak acid are neutralized, a fresh quantity of previously unionized acid is dissociated and this process is continued until all the ions are neutralized.

The acidity of a solution is due to the hydrogen ions that are present. In fact, the definition of an acid is a substance which in aqueous solution dissociates into hydrogen ions. Further, we know that the hydrogen ions of an acid present in water carry a positive charge of electricity, while the acid radicle of the acid carries one or more negative electric charges. Thus, when hydrochloric acid gas is passed into water, it ionizes into hydrogen and chlorine ions, and at equilibrium we have:

$$HCl \rightleftharpoons H^+ + Cl^-$$

Alkalis in aqueous solution also ionize. For example, a solution of sodium hydroxide in water contains at equilibrium, sodium ions, hydroxyl ions and undissociated sodium hydroxide:

$$NaOH \rightleftharpoons Na^+ + OH^-$$

Water also, even the purest water, dissociates to a minute extent into hydrogen and hydroxyl ions:

$$HOH \rightleftharpoons H^+ + OH^-$$

If, now, an acid solution be added to pure water, which is an equilibrium mixture consisting of hydrogen, hydroxyl ions and undissociated molecules of water, many hydrogen ions are being added to this equilibrium mixture and as a result there will be a change in the equilibrium. Some of the hydroxyl ions will combine with the hydrogen ions, and as a result the number of hydroxyl ions will be decreased. The decrease in the number of hydroxyl ions will mean an increase in the number of hydrogen

ions, and the greater the number of hydrogen ions added, the greater will be the decrease in the number of hydroxyl ions, and the solution will become more strongly acid.

In the same way, if an alkali be added to water, the number of hydroxyl ions will be increased and the number of hydrogen ions diminished. The more strongly alkaline the solution becomes the fewer will be the number of hydrogen ions present.

The relationship between the hydrogen ion concentration and hydroxyl ion concentration is governed by the Law of Mass Action, and at equilibrium we may write:

$$\frac{(\text{Concentration of } A^+) \times (\text{Concentration of } B^-)}{(\text{Concentration of unionized } AB)} = k \text{ (a constant)}$$

In the case of water, we have:

$$\frac{(H^+) \times (OH^-)}{(HOH)} = k \text{ (again a constant)}$$

But in the case of water, HOH is enormous compared with the concentration of hydrogen and hydroxyl ions that are present, and may therefore be considered to be a constant. So that we have now:

$$(H^+) \times (OH^-) = k(HOH) = K$$

Extremely careful measurements of K made for water have shown it to have the value $10^{-14 \cdot 14}$. Since in pure water we have an equal number of hydrogen and hydroxyl ions, the ionic concentration must be:

$$(H^+) \times (OH^-) = 10^{-7 \cdot 07} \times 10^{-7 \cdot 07} = 10^{-14 \cdot 14}$$

Pure water will therefore contain in one litre, one ten-millionth of a gram-ion of ionized hydrogen and the same concentration of hydroxyl ions. However strongly alkaline a solution may be made, the equilibrium equation still holds good, so that strongly alkaline solutions have a definite hydrogen ion concentration which we may express by the equation:

$$(H^+) = \frac{10^{-14 \cdot 14}}{(OH^-)}$$

Thus all aqueous solutions have a definite hydrogen ion concentration. The strength of an acid depends upon the concentration of hydrogen ion present in a given volume, while the strength of an alkali depends upon the concentration of hydroxyl

ions present in a given volume. Moreover, we are able from the equation given above to express equally definitely the strength of an alkaline solution in terms of hydrogen ion concentration.

An acid, alkali or salt dissolved in water is only completely dissociated into ions at infinite dilution. Since the effective strength of an acid or alkali depends upon the concentration of hydrogen ions, we must know the degree of dissociation that has occurred in a given solution in order to know the strength of our solution. There are various methods of doing this. For the accurate determination of the hydrogen ion concentration of a solution an electrical method is employed. Another method which is not so accurate, but more readily applied, is to use certain indicators. For the experimental details see Clark, *The Determination of Hydrogen Ions.*

For the sake of convenience it is usual to express the hydrogen ion concentration in the following way. In a decinormal solution of hydrochloric acid, we should have, on the assumption that complete dissociation of the acid has taken place, $0 \cdot 1$ gm. of hydrogen ions in a litre. It has been found, however, by actual measurement, that the concentration of hydrogen ions in a decinormal solution of hydrochloric acid is only 91 per cent of the total. The actual concentration is therefore, $0 \cdot 1 \times 91$. This may be written $9 \cdot 1 \times 10^{-2}$. Then we have $\log_{10} 9 \cdot 1 = 0 \cdot 9590$, and $9 \cdot 1 \times 10^{-2} = 10^{\cdot 9590 - 2} = 10^{-1 \cdot 041}$. It is the custom now to express hydrogen ion concentration as the exponent to the base 10 with the negative sign omitted, and this value is represented by the symbol pH, or $\mathrm{pH} = \log \frac{1}{(\mathrm{H}^+)}$. The pH of a decinormal solution of hydrochloric acid is therefore $1 \cdot 041$. In the same way the pH of a decinormal solution of acetic acid can be obtained. The hydrogen ion concentration is $1 \cdot 3 \times 10^{-3}$. From this we have $\log_{10} 1 \cdot 3 = 0 \cdot 1139$ and $1 \cdot 3 \times 10^{-3} = 10^{\cdot 1139 - 3} = 10^{-2 \cdot 886}$. Hence the $\mathrm{pH} = 2 \cdot 886$. Similarly, if we know the pH of a solution we can readily calculate the hydrogen ion concentration. It has been found by actual determination that the pH of arterial blood is $7 \cdot 33$. From this value it is possible to calculate the hydrogen ion concentration:

$$\mathrm{pH} = 7 \cdot 33 = 8 - 0 \cdot 67 = 8 - \log 4 \cdot 68 = \log \frac{1}{(4 \cdot 68 \times 10^{-8})}$$

and the hydrogen ion concentration is therefore $4 \cdot 68 \times 10^{-8}$.

Very exact determinations have shown that the pH of the purest water is 7·07 for the hydrogen ion concentration is $10^{-7 \cdot 07}$, from this we have the pH = log $1/10^{-7 \cdot 07} = 7 \cdot 07$.

We have already seen that

$$(H^+) \times (OH^-) = K = 1 \times 10^{-14 \cdot 14}$$

Since the concentration of hydrogen and hydroxyl ions is exactly equal it follows that for absolute neutrality the concentration of hydrogen and hydroxyl ions is also exactly equal. From the nature of this equation, it is clear that the higher the acidity, i.e. the higher the concentration of hydrogen ions, the lower is the pH, the extreme values being 0 and 14.

GENERAL BIBLIOGRAPHY

1. ARBER (1925). *Monocotyledons*. Cambridge.
2. ARMSTRONG, E. F., and ARMSTRONG, K. F. (1931). *The Carbohydrates*. London.
3. —— (1931). *The Glycosides*. London.
4. ATKINS (1916). *Some Recent Researches in Plant Physiology*. London.
5. BABCOCK and CLAUSEN (1927). *Genetics in Relation to Agriculture*. New York.
6. BALLS (1913). *The Cotton Plant in Egypt*. London.
7. BARTON-WRIGHT (1933). *Recent Advances in Plant Physiology*. London.
8. BAYLISS (1925). *The Nature of Enzyme Action*. London.
9. —— (1927). *Principles of General Physiology*. London.
10. BRENCHLEY (1927). *Inorganic Plant Poisons and Stimulants*. Cambridge.
11. BURGERSTEIN (1920). *Die Transpiration der Pflanzen*. Jena.
12. CLARK (1928). *The Determination of Hydrogen Ions*. Baltimore.
13. CLEMENTS (1920). *Aeration and Air-Content*. Washington.
14. CURTIS (1935). *Translocation in Plants*. New York.
15. CZAPEK (1905–22). *Biochemie der Pflanzen*. Bd. I, II and III. Jena.
16. DARLINGTON (1937). *Recent Advances in Cytology*. London.
17. DARWIN, C. (1875). *Insectivorous Plants*. London.
18. —— (1880). *The Power of Movement in Plants*. London.
19. DIXON (1914). *Transpiration and the Ascent of Sap*. London.
20. —— (1924). *The Transpiration Stream*. London.
21. DORÉE (1932). *Methods of Cellulose Chemistry*. London.
22. EAST and JONES, E. M. (1919). *Inbreeding and Outbreeding, their Genetic and Sociological Significance*. Philadelphia.
23. FRYER and WESTON (1920). *Oils, Fats and Waxes*. Vols. I and II. London.
24. FUCHS (1926). *Die Chemie des Lignins*. Berlin.
25. GORTNER (1929). *Outlines of Biochemistry*. New York.
26. GRAY (1931). *A Text-book of Experimental Cytology*. Cambridge.
27. HAAS, P., and HILL (1928–29). *An Introduction to the Chemistry of Plant Products*. Vol. I. *On the Nature and Significance of the Commoner Organic Compounds of Plants*. Vol. II. *Metabolic Processes*. London.
28. HABERLANDT (1914). *Physiological Plant Anatomy*. Translated by M. Drummond. London.
29. HALDANE, J. B. S. (1930). *Enzymes*. London.
30. HALDANE, J. S. (1920). *Methods of Air Analysis*. London.
31. HARDEN (1932). *Alcoholic Fermentation*. London.
32. HAWLEY and WISE (1926). *The Chemistry of Wood*. New York.
33. HEILBRUNN (1928). *The Colloid Chemistry of Protoplasm*. Berlin.
34. JAMIESON (1932). *Vegetable Oils and Fats*. New York.
35. JENNINGS (1935). *Genetics*. London.
36. JENSEN (1936). *Growth Hormones in Plants*. Translated by G. S. Avery and P. R. Burkholder. New York.
37. JONES, W. (1920). *Nucleic Acids*. London.
38. JOST (1907). *Lectures on Plant Physiology*. Translated by R. J. Harvey-Gibson. Oxford. (Supplement, 1913. Oxford.)

39. KOSTYTCHEV (1927). *Plant Respiration*. Translated and Edited by C. J. Lyon. Philadelphia.
40. —— (1931). *Chemical Plant Physiology*. Translated and Edited by C. J. Lyon. Philadelphia.
41. KOSTYTCHEV and WENT (1931). *Lehrbuch der Pflanzenphysiologie* Bd. II. *Stoffaufnahme. Stoffwanderung. Wachstum und Bewegungen.* Berlin.
42. LEATHES and RAPER (1925). *The Fats*. London.
43. LEVENE and BASS (1931). *Nucleic Acids*. New York.
44. LLOYD, JORDAN D. (1926). *Chemistry of the Proteins*. London.
45. LUNDEGÅRDH (1931). *Environment and Plant Development*. Translated and Edited by E. Ashby. London.
46. MACLEAN (1927). *Lecithin and Allied Substances*. London.
47. MAXIMOV (1929). *The Plant in Relation to Water*. Edited with notes by R. H. Yapp. London.
48. MILLER (1931). *Plant Physiology*. New York.
49. ONSLOW (1925). *The Anthocyanin Pigments of Plants*. Cambridge.
50. —— (1931). *The Principles of Plant Biochemistry*. Cambridge.
51. —— (1931). *Practical Plant Biochemistry*. Cambridge.
52. OSBORNE (1924). *The Vegetable Proteins*. London.
53. OSTERHOUT (1922). *Injury, Recovery and Death, in Relation to Conductivity and Permeability*. Philadelphia.
54. OSTWALD (1919). *Handbook of Colloid Chemistry*. Translated by M. H. Fisher. London.
55. PALLADIN (1922). *Plant Physiology*. Edited by B. E. Livingston. Philadelphia.
56. PARSONS (1933). *Fundamentals of Biochemistry*. Cambridge.
57. PEARL (1922). *The Biology of Death*. Philadelphia.
58. —— (1928). *The Rate of Living*. London.
59. PFEFFER (1900–3). *The Physiology of Plants*. Vols. I, II and III. Translated by A. J. Ewart. Oxford.
60. PRYDE (1933). *Recent Advances in Biochemistry*. London.
61. PUNNETT (1927). *Mendelism*. London.
62. RAYNER (1927). *Mycorrhiza*. London.
63. RIDEAL (1930). *An Introduction to Surface Chemistry*. Cambridge.
64. ROBERTSON (1923). *The Chemical Basis of Growth and Senescence*. Philadelphia.
65. ROBINSON, G. W. (1936). *Soils, their Origin, Constitution and Classification*. London.
66. RUSSELL (1937). *Soil Conditions and Plant Growth*. London.
67. SACHS (1887). *Lectures on the Physiology of Plants*. Translated by H. Marshall-Ward. Oxford.
68. SCHIMPER (1903). *Plant Geography upon a Physiological Basis*. Oxford.
69. SCHORGER (1926). *The Chemistry of Cellulose and Wood*. New York.
70. SEIFRIZ (1936). *Protoplasm*. New York and London.
71. SKENE, MACGREGOR (1924). *The Biology of Flowering Plants*. London.
72. SPOEHR (1926). *Photosynthesis*. New York.
73. STEEL (1928). *Physical Chemistry and Biophysics*. London.
74. STEELE (1934). *An Introduction to Plant Biochemistry*. London.

75. STEPHENSON (1930). *Bacterial Metabolism*. London.
76. STILES (1924). *Permeability*. London.
77. —— (1925). *Photosynthesis*. London.
78. TAYLOR (1921). *The Chemistry of Colloids*. London.
79. THOMPSON (1917). *Growth and Form*. London.
80. VERWORN (1913). *Irritability*. Oxford.
81. WALDSCHMIDT-LEITZ (1929). *Enzyme Action and Properties*. Translated by R. P. Walton. New York.
82. WARMING (1909). *Ecology of Plants*. Oxford.
83. WAXMAN (1927). *The Principles of Soil Microbiology*. London.
84. WEAVER (1926). *Root Development of Field Crops*. New York.
85. WERNER (1923). *The Chemistry of Urea*. London.
86. WILLSTÄTTER (1928). *Untersuchungen über Enzyme*. Vols. I and II. Berlin.
87. WILLSTÄTTER and STOLL (1918). *Untersuchungen über Chlorophyll*. Berlin.

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